VERIFICATION OF TRANSLATION

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declare as follows:

1. That I am well acquainted with both the English and Japanese languages, and

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[Document Name] Specification

[Title of the Invention] Transporter Genes

[Claims]

[Claim 1] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

[Claim 2] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 3] A protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added.

[Claim 4] A protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 4.

[Claim 5] A DNA encoding the protein according to claim 1 or 3.

[Claim 6] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 2.

[Claim 7] A DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO:4.

[Claim 8] A vector comprising the DNA according to any one of claims 5 to 7.

[Claim 9] A transformant expressibly carrying the DNA according to any one of claims 5 to 7.

[Claim 10] A method for producing the protein according to any one of claims 1 to 4, comprising the step of culturing the transformant according to claim 9.

[Claim 11] An antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:1.

[Claim 12] An antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[Detailed Description of the Invention]

[0001]

[Technical Field of Industrial Application]

The present invention relates to transporters, proteins involved in transport of substances from the outside to the inside of cells or vice versa.

[0002]

[Prior Art]

Recently, the involvement of various transporters localized on the plasmamembrane in the uptake system for nutrients and endogenous substances into cells and their transport mechanisms have been clarified (Tsuji, A. and Tamai, I., Pharm. Res., 13, 963-977, 1996). These transporters recognize the structures of substances to be transported to selectively transport specific substances. Transporters that recognize the relatively wide range of structures may possibly recognize foreign substances such as drugs by mistake, and actively take in them into cells. It is believed that drugs permeate through the plasmamembrane fundamentally by simple diffusion depending on their physicochemical properties such as molecular size, hydrophobicity, and hydrogen-binding capacity. Particularly, in the case of ionic drugs, only molecules in the non-dissociated form can permeate through the plasmamembrane according to the pH partition hypothesis. However, it has become evident that a number of drugs penetrate through the cell membrane by a specific mechanism other than simple diffusion, that is, an active transport mediated by transporters, in organs that require efficient exchange of intracellular and extracellular substances, including small intestine, uriniferous tubule, placenta, epithelial cells of choroid plexus, hepatocytes, and blood-brain barrier (Tamai, I. and Tsuji, A., Pharmacia, 31, 493-497, 1995; Saito, H. and Inui, K., Igaku no Ayumi, 179, 393-397, 1996; Tamai, I., Yakubutsu Dotai (Pharmacokinetics), 11, 642-650, 1996). For example, it is known that although oral β-lactam antibiotics of the non-esterified type are amphoteric or negatively charged in physiological pHs and sparingly soluble in fat, they are readily absorbed through the intestine. The transport study

using the isolated membrane-vesicles system demonstrated that an H^+-driven peptide transporter localized on the brush-border is involved in the absorption process of these drugs (Okano, T. et al., J. Biol. Chem., 261, 14130-14134, 1986). Although the specificity of a peptide transport system in terms of the peptide size is so strict as to recognize di- or tri-peptides but not tetrapeptides or larger peptides, it has a rather broad substrate specificity to recognize peptides comprising non-natural amino acids. The peptide transporter mediates transport of β-lactam antibiotics mistakenly due to its broad substrate specificity. This property has been unexpectedly utilized in the clinical field (Tsuji, A., American Chemical Society (eds. Taylor, M. D., Amidon, G. L.), Washington, D. C., 101-134, 1995). Furthermore, a possibility that a transporter is also involved in permeation of substances with a high hydrophobicity such as fatty acids through the plasmamembrane has been reported (Schaffer, J. and Lodish, H., Cell, 79, 427-436, 1994).

[0003]

Since various transporters are supposed to be distributed in organs and cells based on the physiological roles of the organs and cells, their distribution and functions may be specific to organs. Therefore, transporters are expected to be used to impart an organ specificity to pharmacokinetics. In other words, an organ-specific drug delivery system (DDS) can be constructed utilizing transporters. If drug absorption solely relied on simple diffusion is improved by elevating its hydrophobicity, an effect of the drug obtained in the initial transport in the liver can be enhanced and the drug can non-specifically translocate into any organ. In addition, it would also be possible to increase the drug absorption independently of its fat-solubility by designing the drug utilizing the substrate specificity of transporters (Hayashi, K. et al., Drug Delivery System, 11, 205-213, 1996). For this purpose, it is necessary to identify various transporters at the molecular level and analyze their properties in detail. However, their molecular level identification are greatly behind studies on their membrane physiology because they

are difficult to handle biochemically and require complicated processes in their functional assays.

[0004]

Recently, cDNAs of several transporters have been cloned by the expression cloning method using *Xenopus* oocytes, a foreign gene expression system, and structural homology among them has been revealed (Fei, Y.-J. et al., Nature, 368, 563-566, 1994). For example, Koepsell et al. cloned an organic cation transporter, OCT1, which is assumed to be localized on a basement membrane, using the expression cloning method in 1994 (Grundemann, D. et al., Nature, 372, 549-552, 1994). Subsequently, OCT2 was identified by homology cloning based on the sequence of OCT1 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996). OCT1 and OCT2 show homology as high as 67% to each other, but they differ in their distribution at uriniferous tubules, and OCT2 is believed to be mostly localized at luminal side (Grundemann, D. et al., J. Biol. Chem., 272, 10408-10413, 1997). Both of them are intensely expressed in the kidney, but differ in the organ distribution; OCT1 is also expressed in the liver, colon, and small intestine, while OCT2 expression is specific to the kidney.

[0005]

Only a few reports on identification of transporters at the molecular level, including the reports, are available, and there would be many unknown transporters that may be clinically useful.

[0006]

[Problems to Be Solved by the Invention]

An object of this invention is to provide a novel transporter gene and its protein.

[0007]

[Means to Solve the Problems]

The present inventors have screened a fetal gene library constructed using the subtractive method by random sequencing based on a working hypothesis that fetal genes include those which are involved in various disorders including cancer and are specifically or intensely expressed in fetal tissues. The inventors discovered an unknown gene showing a significant homology with those for organic

cation transporters, OCT1 and OCT2, and attempted to isolate this gene, which was assumed to encode a novel transporter. Thus, the inventors succeeded in isolating the desired gene by screening a cDNA library derived from human fetus. Furthermore, the inventors studied the transporter activity of a protein encoded by the isolated human gene and found that the protein, in fact, functioned as a transporter for various organic cations.

[0008]

This invention relates to a novel transporter, and more specifically to:

(1) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:1 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added;

(2) a protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising a nucleotide sequence set forth in SEQ ID NO: 2;

(3) a protein having an activity to transport an organic cation, comprising an amino acid sequence set forth in SEQ ID NO:3 or said amino acid sequence in which one or more amino acids are substituted, deleted, or added;

(4) a protein having an activity to transport an organic cation, encoded by a DNA hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 4;

(5) a DNA encoding the protein according to (1) or (3);

(6) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO: 2;

(7) a DNA encoding a protein having an activity to transport an organic cation, hybridizing to a DNA comprising an nucleotide sequence set forth in SEQ ID NO:4;

(8) a vector comprising the DNA according to any one of (5) to (7);

(9) a transformant expressibly carrying the DNA according to any one of (5) to (7);

(10) a method for producing the protein according to any

one of (1) to (4), comprising the step of culturing the transformant according to (9);

(11) an antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:1; and

(12) an antibody that binds to a protein comprising an amino acid sequence set forth in SEQ ID NO:3.

[0009]

[Mode for Carrying Out the Invention]

This invention relates to a novel transporter protein. Nucleotide sequences of cDNAs of novel human transporters isolated by the present inventors are shown in SEQ ID NO: 2 and SEQ ID NO: 4, respectively. Amino acid sequences of proteins encoded by these cDNAs are shown in SEQ ID NO: 1 and SEQ ID NO: 3, respectively. Amino acid sequences of these two proteins included in the transporter proteins of this invention showed such a high overall homology as about 76%, and both of them retained the following consensus sequence which is conserved in various types of transporters including the glucose transporter: [Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala] (Maiden, M. C. et al., Nature, 325, 641-643, 1987). In fact, these proteins have an activity to transport various organic cations.

[0010]

Transporter proteins of this invention also include those having the additional activity to transport substances other than organic cations as far as they retain the organic cation transport activity. Organic cations include, for example, TEA, carnitine, quinidine, and pyrilamine, but are not limited to them. They also include carcinostatic agents such as actinomycin D, etoposide, vinblastine, daunomycin, etc. Transporter proteins of this invention include those having the activity to transport organic cations not only from the outside to the inside of cells but also from the inside to the outside of cells.

[0011]

Transporter proteins of this invention can be prepared as recombinant proteins using recombination techniques or natural proteins. Recombinant proteins can be prepared, for example, as described below, by culturing cells transformed with DNA encoding proteins of this invention. Natural proteins can be isolated from the kidney and cancer cell strains such as Hela S3, which highly express the proteins of this invention, by the method well known to those skilled in the art, for example, affinity chromatography using an antibody of this invention described below. The antibody may be either polyclonal or monoclonal. A polyclonal antibody can be prepared by purifying serum obtained from, for example, a small animal such as a rabbit immunized with proteins of this invention by known methods, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. A monoclonal antibody can be prepared by immunizing a small animal such as a mouse with the protein of this invention, excising the spleen from the mouse, grinding the tissue into cells, fusing them with mouse myeloma cells using a fusing agent such as polyethylene glycol, and selecting a clone that produces an antibody to proteins of this invention out of fused cells (hybridomas) thus produced. Then, hybridomas thus selected are transplanted into the abdominal cavity of a mouse, and the ascites is collected from the mouse. A monoclonal antibody thus obtained can be purified by, for example, ammonium sulfate precipitation, protein A or protein G column, DEAE-ion exchange column chromatography, affinity column chromatography coupled with the protein of this invention, etc. When the antibody thus obtained is administered to human subjects, a humanized antibody or a human antibody is advantageously used to reduce the immunogenicity. An antibody can be humanized by, for example, the CDR grafting method comprising cloning an antibody gene from monoclonal antibody-producing cells and grafting the epitope portion thereof into an existing human antibody. A human antibody can be prepared by the usual method for preparing a monoclonal antibody except for immunizing a mouse whose immune system is replaced with the

human's.

[0012]

It is also possible for those skilled in the art to prepare proteins having functions equivalent to the transporter proteins set forth in SEQ ID NOs: 1 or 3 by appropriately modifying amino acid residues of the proteins by, for example, substitution, using well known methods. Mutation of amino acids of the proteins can occur also spontaneously. Such mutant proteins which are obtained by altering the amino acid sequence of the proteins set forth in SEQ ID NOs: 1 or 3 by substitution, deletion, or addition of amino acid residues, and are functionally equivalent to those of the proteins set forth in SEQ ID NOs: 1 or 3 are also included in the proteins of this invention. Herein, "functionally equivalent" means that proteins have an activity to transport organic cations. Methods well known to those skilled in the art for altering amino acids include, for example, the site-specific mutagenesis system by PCR (GIBCO-BRL, Gaithersburg, Maryland), site-specific mutagenesis by oligonucleotide (Kramer, W. and Fritz, H. J. (1987) Methods in Enzymol., 154: 350-367), Kunkel's method (Methods Enzymol., 85, 2763-2766 (1988)), etc. The number of amino acids that can be substituted is usually 10 amino acid residues or less, preferably 6 or less, and more preferably 3 or less. The site of substitution, deletion, or addition of amino acid residues is not particularly limited as far as the activity of proteins of this invention is retained. It is possible to detect the transporter activity of proteins, for example, by the method described below in Example 6.

[0013]

It is routine for those skilled in the art to obtain proteins functionally equivalent to the proteins of this invention by isolating and using DNAs highly homologous to the DNA sequences set forth in SEQ ID NOs: 2 or 4 or portions thereof using hybridization techniques (Sambrook, J. et al., Molecular Cloning 2nd ed., 9.47-9.58, Cold Spring Harbor Lab. press, 1989), etc. Therefore, it is possible for those skilled in the art to prepare proteins which are coded by the DNA hybridizing to DNA of SEQ ID NOs:2 or

4 and are functionally equivalent to the transporter proteins of SEQ ID NOs: 1 or 3. These proteins are also included in proteins of this invention. Here, "functionally equivalent" means that proteins have an activity to transport organic cations, as mentioned above. DNAs that hybridize to the DNAs encoding the proteins of this invention can be isolated from other organisms, for example, mice, rats, rabbits, cattle, etc. Especially, tissues such as the kidney are suitable as sources of such DNAs. These DNAs thus isolated which encode the proteins having equivalent function to the proteins set forth in SEQ ID NOs: 1 or 3 usually have a high homology with the DNAs set forth in SEQ ID NOs: 2 or 4. "High homology" means at least 70% or more, preferably at least 80% or more, and more preferably at least 90% or more of sequence identity with DNAs set forth in SEQ ID NOs: 2 or 4.

[0014]

One example of hybridization conditions for isolating such DNAs is as follows. That is, after the pre-hybridization at 55℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 37℃ to 55℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS once at 37℃ for 20 min. More preferable conditions are as follows. After the pre-hybridization at 60℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 60℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 1 x SSC and 0.1% SDS twice at 50℃ for 20 min. Still more preferable conditions are as follows. After pre-hybridization at 68℃ for 30 min or more in the "ExpressHyb Hybridization Solution" (CLONTECH), a labeled probe is added, and hybridization is performed by heating the reaction mixture at 68℃ for 1 h or more. Then, the reaction product is successively washed in 2 x SSC and 0.1% SDS three times at room temperature for 20 min, and then in 0.1 x SSC and 0.1%

SDS twice at 50℃ for 20 min.

[0015]

The present invention also relates to DNAs encoding the above-described transporter proteins of this invention. DNAs of this invention may be cDNA, genomic DNAs, and synthetic DNAs. The DNAs of the present invention can be used for producing proteins of this invention as recombinant proteins, as well as for isolating proteins which are functionally equivalent to proteins of SEQ ID NOs:1 or 3 . That is, it is possible to prepare proteins of this invention as recombinant proteins by inserting DNAs encoding proteins of this invention (e.g. DNAs set forth in SEQ ID NOs: 2 or 4) into an appropriate expression vector, culturing transformants obtained by transfecting suitable cells with the vector, and purifying the proteins thus expressed. Cells to be used for producing recombinant proteins include, for example, mammalian cells such as COS cells, CHO cells, NIH3T3 cells, etc., insect cells such as Sf9 cells, yeast cells, *E. coli*, and so on. Vectors used for the intracellular expression of recombinant proteins vary depending on host cells, including, for example, pcDNA3 (Invitrogen), pEF-BOS (Nucleic Acids Res., 1990, 18(7), p5322), etc. for mammalian cells, "BAC-to-BAC baculovirus expression system" (GIBCO BRL), etc. for insect cells, "Pichia Expression Kit" (Invitrogen), etc. for yeast cells, pGEX-5X-1 (Pharmacia), "QIAexpress system" (Qiagen), etc. for *E. coli*. Host cells can be transformed with vectors, for example, by the calcium phosphate method, the DEAE-dextran method, the method using cationic liposome DOTAP (Boehringer Mannheim), the electroporation method, the calcium chloride method, etc. Recombinant proteins can be purified from recombinants thus obtained using standard methods, for example, as described in "The Qiaexpressionist Handbook, Qiagen, Hilden, Germany."

[0016]

The DNAs of this invention can be used in gene therapy for disorders caused by abnormalities in the activity and expression of the proteins of this invention. In this case, the DNAs of this invention are inserted into an adenovirus vector (e.g. pAdexLcw),

a retrovirus vector (e.g. pZIPneo), etc., and administered into the living body by either *ex vivo* method or *in vivo* method. Gene therapy can also be performed by administering a synthetic antisense DNA to the living body directly or after inserted into the above-described vectors.

[0017]

The transporter proteins of this invention can be used to control internal absorption and dynamics of drugs. Based on the results of detailed analysis of the substrate specificity of transporter proteins of this invention, drugs can be designed so as to be transported by these transporters and absorbability of the drugs mediated by these transporter proteins can be improved. Conventional modifications to enhance hydrophobicity are no longer necessary for drugs so designed, which enables speedily and efficiently developing water-soluble drugs that are easy to handle. The drugs thus developed are thought to be absorbed principally depending on the internal distribution pattern of transporter proteins of this invention, and an organ-specific delivery of the drugs thus becomes possible. Especially, if the transporter proteins of this invention are distributed in the target organ of a drug, an ideal drug delivery system (DDS) can be developed. If a drug is to be absorbed mediated by not the transporter proteins of this invention but other transporters, the drug can be designed so as to be specific to other transporter proteins by designing it considering the substrate specificity of the transporter proteins of this invention. Since the transporter proteins of this invention are present in the kidney, it is possible to reduce the nephrotoxicity produced by a drug by designing the drug so that it can be readily excreted by the transporter proteins of this invention.

[0018]

Another possible application of this invention is to develop a drug targeting the transporter proteins of this invention. The transporters play important roles in the absorption mechanism of nutrients and drugs, or the excretion mechanism of drugs and internal metabolites. Thus, damage or abnormal elevation of the

transporter's functions may cause some disorders. It is considered to be efficacious against such disorders to administer a drug that inhibits or enhances functions of the transporter proteins of this invention, or regulates the expression level of the transporter gene of this invention and the amount of the transporter proteins. The gene therapy above-mentioned is also efficacious.

[0019]

Furthermore, since "OCTN2" included in the transporter proteins of this invention efficiently transports carnitine, chemotherapy with compounds to control the activity of "OCTN2" or gene therapy using the "OCTN2" gene is considered to be efficacious against various pathological conditions such as fatty liver, etc. caused by hypocarnitinemia.

[0020]

The transporter proteins of this invention are expressed in a variety of cancer cell strains, which suggests that the proteins may transport drugs into tumor cells. If this is the case, it is possible to develop carcinostatics that will be readily absorbed mediated by the transporter proteins of this invention. On the contrary, mechanisms to transport and excrete substances by the transporter proteins of this invention may function to excrete carcinostatics in tumor cells so that the cells acquire resistance to drugs. If the transporter proteins of this invention are involved in a mechanism of tumor cells to acquire drug resistance, a carcinostatic effect can be enhanced by a combined use of inhibitors of the transporter proteins of this invention with carcinostatics.

[0021]

The present invention is described below in more detail with reference to examples, but is not construed being limited thereto.

[0022]

[Examples]

Example 1 Construction of a subtraction library

A subtraction library was constructed using the PCR-Select™ cDNA Subtraction Kit (CLONTECH) principally according to the method of Luda Diatchenko (Diatchenko, L. et al., Proc. Natl. Acad. Sci.

USA, 93, 6025-6030, 1996).

[0023]

First, double-stranded cDNAs were synthesized from poly(A)$^+$ RNAs derived from human fetal liver and adult liver by the standard method using MMLV reverse transcriptase. These cDNAs were blunt-ended with T4 DNA polymerase and cleaved with RsaI. A part of the cDNAs derived from fetal liver (tester) was divided in two portions, and they were separately ligated to two different adapters, adapter 1 and adapter 2, respectively (Table 1). A 120-fold excess of cDNA derived from adult liver (driver) was added to each of the above-described tester samples. The mixture was heat-denatured and subjected to the primary hybridization at 68℃ for 8 h. After these two reaction mixtures from the primary hybridization were mixed together without heat-denaturation, an excessive amount of the heat-denatured driver was further added thereto, and the mixture was subjected to the secondary hybridization at 68℃ for about 16 h. The resulting reaction solution was diluted with a dilution buffer and incubated at 75℃ for 7 min. After the shorter strands of adapters were removed, the reaction solution was used as a template for PCR. PCR using primers 1 (5'-CTAATACGACTCACTATAGGGC-3', SEQ ID NO: 5) and 2 (5'-TGTAGCGTGAAGACGACAGAA-3', SEQ ID NO: 6) corresponding to the adapters selectively amplified only cDNAs having different adapters at their both ends (subtracted cDNAs)(suppression PCR). PCR was carried out using a portion of the resulting cDNA as a template, and nested PCR primers 1 (5'-TCGAGCGGCCGCCCGGGCAGGT-3', SEQ ID NO: 7) and 2 (5'-AGGGCGTGGTGCGGAGGGCGGT-3', SEQ ID NO: 8), which are further inwardly located from the PCR primers 1 and 2, to obtain products with further elevated selectivity. PCR products thus obtained were purified using the QIAquick PCR Purification kit (QIAGEN), and cloned into the pT7Blue-T vector (Novagen) by the TA cloning method to construct a subtraction library.

[0024]

[Table 1]

Adapter 1	5'-CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT-3' 3'-GGCCCGTCCA-5'

Adapter 2	5'-TGTAGCGTGAAGACGACAGAAAGGGCGTGGTGCGGAGGGCGGT-3' 3'-GCCTCCCGCCA-5'

Example 2 cDNA cloning

To analyze fetal genes, the subtraction library derived from the fetal liver was screened by random sequencing. Homology search (Blastx) of Expressed Sequence Tags (ESTs) thus obtained found a clone, OCTN1 (fls 631) (292 bp) encoding amino acid sequence having significant homology with the known organic cation transporters, OCT1 (Grundemann, D. et al., Nature, 372, 549-552, 1994) and OCT2 (Okuda, M. et al., Biochem. Biophys. Res. Commun., 224, 500-507, 1996). Since the sequence of this clone was novel and assumed to be a fragment derived from a new transporter gene, cDNA comprising the whole open reading frame (ORF) of this gene was cloned.

[0025]

The human fetal liver 5'-stretch cDNA library (CLONTECH) was screened using the original OCTN1 clone obtained from the subtraction library derived from fetal liver as a probe. An insert of the original OCTN1 clone was amplified by PCR using M13 P4-22 and M13 P5-22, and labeled with [α-^{32}P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was carried out at 68℃ in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50℃ in 0.1 x SSC and 0.1% SDS. Screening about 5 x 10^5 phage clones finally isolated seven positive clones. cDNA inserts of these clones were amplified by PCR using vector primers designed based on a sequence of the λ gt10 vector (GT10 S1 5'-CTTTTGAGCAAGTTCAGCCT-3', SEQ ID NO: 9, and GT10 A1 5'-AGAGGTGGCTTATGAGTATTTCTT-3', SEQ ID NO: 10), or primers designed based on the decoded cDNA sequences. The PCR products thus obtained were directly sequenced to determine the nucleotide sequences. Some regions that were difficult to be amplified were subjected to PCR using 7-deaza dGTP as a substrate base (McConlogue, L. et al., Nucleic Acids Res., 16, 9869, 1988).

[0026]

After screening about 5 x 10^5 phage clones, finally seven positive clones were isolated. Sequencing of cDNA inserts of these clones revealed that the OCTN1 gene contains an ORF encoding a protein consisting of 551 amino acid residues (putative molecular weight of about 62,000). Data base search using this whole amino acid sequence confirmed that it has a significant overall homology (about 34%) with OCT1 and OCT2. Hydrophobicity profile of this sequence obtained by Kyte & Doolittle's calculating formula (Kyte, J. and Doolittle, R. F., J. Mol. Biol., 157, 105-132, 1982) very closely resembled those of OCT1 and OCT2, indicating that the sequence has eleven to twelve putative transmembrane hydrophobic regions (Fig. 1). This sequence contained one consensus sequence of sugar transporter, ([Leu, Ile, Val, Met, Ser, Thr, Ala, Gly]-[Leu, Ile, Val, Met, Phe, Ser, Ala, Gly]-Xaa<2>-[Leu, Ile, Val, Met, Ser, Ala]-[Asp, Glu]-Xaa-[Leu, Ile, Val, Met, Phe, Tyr, Trp, Ala]-Gly-Arg-[Arg, Lys]-Xaa<4-6>-[Gly, Ser, Thr, Ala]), (160 to 175). This consensus sequence is present in the glucose transporters GLUT1 to GLUT7 in mammalian cells, and also present in various types of transporters other than glucose transporters (Maiden, M. C. et al., Nature, 325, 641-643, 1987). Furthermore, putative N-linked glycosylation sequences (N-X-[ST]) were found in the amino acid sequence of OCTN1 at four sites (57 to 59, 64 to 66, 91 to 93, and 304 to 306), and also five putative protein kinase C phosphorylation sites ([ST]-X-[RK]) (164 to 166, 225 to 227, 280 to 282, 286 to 288, and 530 to 532). In addition, the consensus sequence ([Ala, Gly]-Xaa(4)-Gly-Lys-[Ser, Thr]) of the ATP/GTP binding site is also found. This consensus sequence of the ATP/GTP binding site is also present in the ATP binding protein or GTP binding protein, such as kinases and ras family proteins, and that ATP or GTP binds to this site (Walker, J. E. et al., EMBO J., 1, 945-951, 1982). This sequence is present in the so-called ATP Binding Cassette (ABC) type transporter, and involved in the substance transport using the energy generated by hydrolysis of ATP (Higgins, C. F. et al., J. Bioenerg. Biomembr., 22, 571-592, 1990; Urbatsch, I. L. et al., J. Biol. Chem., 270, 26956-26961,

1995). Presence of this consensus sequence indicates that OCTN1 protein may be an ATP or GTP-dependent transporter.

[0027]

Nucleotide sequencing was performed by the cycle-sequencing method with a plasmid DNA prepared by the alkaline-SDS method or a PCR product obtained by colony PCR, etc. as a template using the ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit With AmplyTaq DNA Polymerase, FS, followed by decoding with the ABI 377 DNA Sequencer (Perkin Elmer). Colony PCR was carried out by directly suspending a colony of a recombinant in a PCR reaction solution containing vector primers M13 P4-22 (5'-CCAGGGTTTTCCCAGTCACGAC-3', SEQ ID NO: 11) and M13 P5-22 (5'-TCACACAGGAAACAGCTATGAC-3', SEQ ID NO: 12). After the completion of PCR, a DNA insert thus amplified was separated from unreacted primers and nucleotides by gel filtration, etc. to serve as a template for sequencing.

[0028]

Example 3 Northern analysis

Distribution of OCTN1 in tissues was investigated by Northern analysis (Fig. 2). A 3'-end fragment of OCTN1 (the latter half from around the base 1,100) was labelled with [α-^{32}P]dCTP by the random primer method using the Ready-to Go DNA labeling beads (Pharmacia) to serve as a probe. Hybridization was performed using the Multiple Tissue Northern (MTN) Blot - Human, Human III, Human IV, Human Fetal II, and Human Cell lines (CLONTECH) at 68℃ in the ExpressHyb Hybridization Solution (CLONTECH) according to the method recommended by the manufacturer. Final washing was performed at 50℃ in 0.1 x SSC and 0.1% SDS. As a result, RNA of about 2.5 kb was strongly expressed in the fetal liver and adult-derived tissues such as the kidney, bone marrow, and trachea. Besides those tissues, the RNA band was also weakly detected in the fetal kidney and lung, and adult tissues including skeletal muscle, lung, placenta, prostate, spleen, and spinal cord. The RNA expression was also detected in tumor cell lines such as HeLa S3, K562, SW480, and A549, and especially, its very intense expression was observed in HeLa S3.

[0029]

Example 4 Cloning of OCTN2 cDNA

Data base search using the entire nucleotide sequence of "OCTN1" detected very similar sequences thereto in several parts of the nucleotide sequence of P1 phage clones (P1 H24 clones, GenBank accession No. L43407, L43408, L46907, L81773, and L43409) derived from q regions of human chromosome 5. The parts having similarity with the nucleotide sequence of OCTN1 are separated by the sequences having no similarity to the OCTN1 sequence. The sequence obtained by connecting these similar parts with each other with reference to the sequence of OCTN1 has a high homology over a wide range with OCTN1, indicating the presence of OCTN1 homologues. The genomic sequence registered in data base was an incomplete one without covering the entire coding region, and, from only this sequence, it was impossible to know the complete structure of a protein partially encoded by the sequence. Therefore, cDNA cloning of this OCTN1 homologous gene (OCTN2) was performed to determine the coded protein structure. First, 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) were prepared based on sequences of these P1 phage clones. PCR was performed using a set of these primers and cDNA synthesized from poly(A)$^+$ RNA derived from the human adult kidney (CLONTECH) as a template, under the following conditions: 1 cycle of 94℃ for 3 min; 35 cycles of 94℃ for 30 s, 58℃ for 1 min, and 72℃ for 2 min,; and 1 cycle of 72℃ for 10 min, resulting in amplification of about 900 bp fragment. This fragment was subcloned into the pT7Blue-T vector (Novagen) by the TA cloning method to determine its nucleotide sequence, which clearly showed a very high overall homology with OCTN1. Therefore, this gene was designated as OCTN2, and longer cDNAs were cloned.

[0030]

The cDNA library derived from the human kidney was screened using the cDNA insert of this clone as a probe in the same manner as for OCTN1 cDNA cloning, and cDNA containing the entire coding region of OCTN2 was cloned by a procedure for isolating longer

clone and the Rapid Amplification of cDNA Ends (RACE) method (Chenchik, A., Moqadam, F., and Siebert, P. (1995), CLONTECHniques X, 5-8), etc. to determine its structure (SEQ ID NO: 4). Specifically, the RACE method was carried out as follows. The 631R S6 primer (5'-AGCATCCTGTCTCCCTACTTCGTT-3', SEQ ID NO: 15) was prepared. PCR was performed using this primer and the Marathon-Ready™ cDNA derived from the human adult kidney (CLONTECH) as a template under the following conditions: 94℃ for 2 min; 35 cycles of 94℃ for 30 s, 60℃ for 1 min, and 72℃ for 3 min,; and 72℃ for 10 min, resulting in amplification of about 1.7 kbp cDNA fragment of the 3'-end. This fragment was subcloned into the pT7Blue-T vector by the TA cloning method to determine its structure.

[0031]

It became evident that OCTN2 contains an open reading frame (ORF) encoding a protein consisting of 557 amino acid residues. Fig. 3 compares amino acid sequences of OCTN1 and OCTN2. Both showed overall amino acid homology as high as about 76%. In addition, one consensus sequence (160 to 176) of sugar transporter was present in the amino acid sequence of OCTN2 like OCTN1. These facts indicated that OCTN2 can be a novel transporter that is structurally related to OCTN1. Furthermore, a consensus sequence (218 to 225) of the ATP/GTP binding site was also present in the amino acid sequence of OCTN2 like in OCTN1.

[0032]

Example 5 Northern analysis

Northern analysis was performed using about 900 bp OCTN2 cDNA as a probe which was obtained by PCR with a set of 631R S4 primer (5'-GTGCTGTTGGGCTCCTTCATTTCA-3', SEQ ID NO: 13) and 631R A1 primer (5'-AGCTGCATGAAGAGAAGGACACTG-3', SEQ ID NO: 14) in the same manner as for OCTN1. The results are shown in Fig. 4. Although the expression pattern of OCTN2 partly overlapped with that of OCTN1, OCTN2 differs from OCTN1 in that the former was very intensely expressed in the kidney among fetal tissues, while the latter was strongly expressed also in cancer cell strains such as K-562, HeLa S3, SW480, etc. as well as the kidney, indicating that OCTN1 and OCTN2 may be involved in transport of substances such as

carcinostatics in these cancer cells.

[0033]

Example 6 Forced expression of OCTN1 in human fetal kidney cells (HEK293) and its activity determination

Phage DNAs were extracted from positive phage clones obtained by screening the clones by the plaque hybridization method using the QIAGEN Lambda Kit (QIAGEN). After the DNA insert was subcloned into the pUC18 vector, cDNA containing the entire ORF which was cleaved out with SmaI and EcoRI was integrated between the EcoRI site and the blunted HindIII site of an expression vector for mammalian cells, pcDNA3 (Invitrogen), to obtain an expression plasmid DNA, pcDNA3/OCTN1. Plasmid DNA was prepared by alkaline-SDS method using the QIAGEN PLASMID MAXI Kit (QIAGEN).

[0034]

The human fetal kidney-derived cell strain, HEK 293 cells were transfected with the plasmid pcDNA3/OCTN1 and pcDNA3 vector containing no insert as a control by the calcium phosphate method. First, the plasmid DNA (10 μg), a Hepes buffer solution (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM Dextrose, and 21 mM Hepes pH 7.1) (1 ml), and 2M $CaCl_2$ (62.5 μl) were combined and allowed to stand at room temperature for 30 min or more to form calcium phosphate coprecipitates. After cells were plated on 10-cm plates at 1.5 x 10^6 cells per plate and cultured for 24 h, the calcium phosphate coprecipitates were added thereto, and the cells were further cultured for 24 h. Then, plates were washed with phosphate buffered saline (PBS), and the cells were further cultured for 24 h after the addition of fresh culture medium.

[0035]

Transport experiment was performed using cells transfected with the plasmid DNA or untreated cells according to the following procedures. Cells were detached from plates using a rubber policeman, suspended in a transport buffer (containing 125 mM NaCl, 4.8 mM KCl, 5.6 mM (+)-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 25 mM Hepes pH 7.4), and pre-incubated for 20 min. An appropriate amount of each labeled substrate ([^{14}C]TEA (tetraethylammonium) (NEN), [^{3}H]carnitine (L-carnitine

hydrochloride) (Amersham), [^{3}H]PCG (benzylpenicillin) (Amersham), [^{3}H]quinidine (ARC), or [^{3}H]pyrilamine (mepyramine) (Amersham)) was then added to the cell suspension, and the resulting mixture was incubated at 37℃ for a predetermined period of time. Incubated cells were overlaid on a silicon layer prepared by layering a mixture of silicon oil and liquid paraffin (specific gravity = 1.022) on a 3 M KCl layer, and separated by centrifugation. Radioactivity of cells was measured to determine the into-the-cell transport activity. In this case, 1 x 10^6 cells were used as one point of cells. HEK 293 cells were cultured in Dulbecco's MEM containing 10% fetal calf serum (FCS) in an atmosphere of 5% carbon dioxide at 37℃.

[0036]

First, the transporter capacity was measured in the cells transfected with pcDNA3/OCTN1 and untreated cells using TEA as a substrate (Fig. 5). A reaction time-dependent TEA uptake into the OCTN1-transfectd cells was clearly observed. This uptake was not observed in untreated cells. Next, effects of the addition of unlabeled TEA on the labeled substrate uptake in this system (cold inhibition) were examined (Fig. 6). A decrease in the apparent uptake of the labeled substrate was clearly seen depending on the concentration of cold TEA added. In this experiment, almost no uptake of the substrate into cells was observed in cells transfected with the pcDNA3 vector containing no insert (Mock) used as a control like in untreated cells used, clearly indicating that this uptake phenomenon is due to the transfection of the cells with OCTN1. Next, to obtain the Km (Michaelis constant) value of OCTN1 to TEA, the uptake of ^{14}C-TEA with various concentrations was measured (Fig. 7). From Lineweaver-Burk reciprocal plot of the net uptake obtained by subtracting the amount of the uptake in Mock cells from that in the human OCTN1-transfected cells, the Km value of 0.44±0.04 mM was obtained with the maximal velocity, Vmax of 6.68±0.34 (nmol/3 min/mg). Next, the transport capacity of OCTN1 for other substrate than TEA was examined (Fig. 8). When the transport capacity was measured using labeled organic cations such as labeled carnitine, quinidine, and pyrilamine, a significant

increase in the uptake of these compounds was clearly observed in OCTN1-transfected cells as compared with Mock cells, clearly indicating that these organic cations can serve as substrates for OCTN1. However, no significant increase in the uptake of an organic anion, PCG (benzylpenicillin), was observed.

[0037]

Example 7 Activity measurement of OCTN1 using *Xenopus* oocytes

cRNA was synthesized *in vitro* using T7 RNA polymerase with pcDNA3/OCTN1 as a template. This cRNA was diluted to the concentration of 0.3 ng/nl, and its 50-nl (15 ng) aliquot was injected into a single oocyte. As a control, 50 nl of distilled water was injected. These oocytes were cultured for 3 days, and then used for the transport experiment. After being preincubated in an uptake buffer (0.05% Tween 80, 100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes pH 7.4) at 25℃ for 20 min, the oocytes were transferred to the uptake buffer containing 0.5 ml of labeled substrate to initiate the uptake. After the incubation at 25℃ for 1 h, the oocytes were washed in the ice-cold uptake buffer three times to terminate the reaction. The oocytes were solubilized in 5% SDS and mixed with Cleasol I (a cocktail for liquid scintillation counter) (3 ml) to determine the radioactivity. The radioactivity of the uptake buffer which contained the labeled compound at the time of incubation (external solution) (10 μl) was also similarly measured. The ratio of the radioactivity (dpm value) in the oocytes to that (dpm value) in the external solution was used as the uptake activity.

[0038]

OCTN1 also expresses the transport capacity for organic cations such as quinidine, mepyramine and carnitine, as well as TEA in this transport experiment system using *Xenopus* oocytes (Fig. 9).

[0039]

Next, the transport capacity of OCTN1 for carcinostatics, etc. was examined. The results revealed that human OCTN1 has the activity to transport actinomycin D, etoposide, vinblastine, and daunomycin (Fig. 10). These results strongly indicate that OCTN1

would be involved in the into-the-cell translocation mechanism (mechanism for absorption by cells) for these drugs, which have been clinically used as carcinostatics. By designing and screening drugs utilizing the substrate specificity of OCTN1 so as to be readily recognized by this transporter, it would be possible to efficiently develop useful drugs that can be readily absorbed by the cells.

[0040]

Example 8 Forced expression of OCTN2 in HEK cells and its activity measurement

The expression plasmid DNA for OCTN2 in mammalian cells was prepared as follows.

A single-stranded cDNA was synthesized from poly(A)$^+$ RNA derived from the human fetal kidney (CLONTECH) using the SuperScript™ II reverse transcriptase (GIBCO BRL). PCR was performed using the thus-obtained cDNA as a template under the following conditions to amplify 5'- and 3'-end fragments of human OCTN2.

[0041]

For the amplification of 5'-end fragment (about 800 bp) of OCTN2, OCTN2 3 primer (5'-GATGGATCCCGGACGGTCTTGGGTCGCCTGCTG-3', SEQ ID NO: 16) and OCN2 4 primer (5'-GATGGATCCAAATGCTGCCACATAGTTGGAGAT-3', SEQ ID NO: 17) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (150 μM 7-deaza dGTP, 50 μM dGTP, 200 μM dATP, 200 μM dTTP, and 200 μM dCTP) according to the following conditions: 94℃ for 2 min; 35 cycles of 94℃ for 30 s, 63℃ for 1 min, and 72℃ for 2 min,; and 72℃ for 10 min. For the amplification of 3'-end fragment (about 1.2 kbp) of OCTN2, OCTN2 7 primer (5'-GATGGATCCATGGGCATGCAGACAGGCTTCAGC-3', SEQ ID NO: 18) and OCTN2 8 primer (5'-GATGGATCCTTCCTCTTCAGTTTCTCCCTTACT-3', SEQ ID NO: 19) were used. PCR was carried out using DNA polymerase ExTaq (TaKaRa) and dNTPs (200 μM dGTP, 200 μM dATP, 200 μM dTTP, and 200 μM dCTP) according to the following conditions: 94℃ for 2 min; 35 cycles of 94℃ for 30 s, 63℃ for 30 s, and 72℃ for 2 min,; and 72℃ for 10 min.

[0042]

These fragments were respectively electrophoresed on agarose gel, excised from the gel, purified, and subcloned into the pT7Blue-T vector. Clones having no PCR error were selected by sequencing, and clones from both fragments were ligated at the PstI site in the overlapping region. Each ligated fragment was eventually incorporated into the BamHI site of the pcDNA3 vector, and used as the expression plasmid DNA pcDNA3/OCTN2.

[0043]

HEK cells were transfected with pcDNA3/OCTN2, the pcDNA3 vector containing no insert (Mock), or pcDNA3/OCTN1 by the method described in Example 6 to perform transport experiments. It was proved that OCTN2 has a high capacity to efficiently transport carnitine (Fig. 11). On the other hand, OCTN2 hardly transported TEA, which were efficiently transported by OCTN1, revealing that they clearly differ in their substrate specificities.

[0044]

Next, Na^+ dependence of OCTN2-mediated carnitine transport was examined using a transport buffer in which Na^+ was replaced with K^+ (Fig. 12). The result showed that carnitine transport mediated by OCTN2 completely depended on the presence of Na^+, indicating that OCTN2 is a symport type transporter that transports substrates and Na^+ in the same direction.

[0045]

[Effects of the Invention]

This invention provides a novel organic cation transporter gene and proteins. Transporter of this invention are useful for developing newly designed drugs that can be transported mediated by these proteins, and pharmaceuticals for disorders caused by functional abnormalities of the proteins.

[Sequence Listing]

(1) Name of applicant: CHUGAI RESEARCH INSTITUTE FOR MOLECULAR MEDICINE, INC.

(2) Title of the invention: Transporter Genes

(3) Reference number: C2-906DP1

(4) Application number:

(5) Filing date:

(6) Country where the priority application was filed and the application number of the application:

Japan, No. Hei 9-260972

(7) Priority date: September 8, 1997

(8) Number of sequences:

SEQ ID NO: 1

SEQUENCE LENGTH: 551

SEQUENCE TYPE: amino acid

TOPOLOGY: linear

MOLECULE TYPE: protein

SEQUENCE

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                 30                  35                  40
Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn
             45                  50                  55
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
         60                  65                  70
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile
     75                  80                  85
Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu
 90                  95                  100                 105
Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln
                110                 115                 120
Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu
            125                 130                 135
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155 160 165
Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe
170 175 180 185
Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val
190 195 200
Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu
205 210 215
Gly Thr Glu Ile Leu Gly Lys Ser Val Arg Ile Ile Phe Ser Thr Leu
220 225 230
Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe
235 240 245
Ala Tyr Phe Ile Arg Asp Trp Arg Met Leu Leu Leu Ala Leu Thr Val
250 255 260 265
Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro
270 275 280
Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile
285 290 295
Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe
300 305 310
Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile
315 320 325
Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser
330 335 340 345
Leu Leu Leu Trp Met Leu Thr Ser Val Gly Tyr Phe Ala Leu Ser Leu
350 355 360
Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser
365 370 375
Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg
380 385 390
Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly
395 400 405
Gly Val Leu Leu Phe Ile Gln Leu Val Pro Val Asp Tyr Tyr Phe Leu
410 415 420 425

Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
430 435 440
Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
445 450 455
Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile
460 465 470
Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
475 480 485
Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
490 495 500 505
Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
510 515 520
Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
525 530 535
Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
540 545 550

SEQ ID NO: 2
SEQUENCE LENGTH: 2135
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULE TYPE: cDNA to mRNA
FEATURE
NAME/KEY: CDS
LOCATION: 147.. 1799
IDENTIFICATION METHOD: E
SEQUENCE

CCCCGGCTTC GCGCCCCAAT TTCTAACAGC CTGCCTGTCC CCCGGGAACG TTCTAACATC 60
CTTGGGGAGC GCCCCAGCTA CAAGACACTG TCCTGAGAAC GCTGTCATCA CCCGTAGTTG 120
CAAGTTTCGG AGCGGCAGTG GGAAGC ATG CGG GAC TAC GAC GAG GTG ATC GCC 173
Met Arg Asp Tyr Asp Glu Val Ile Ala
1 5
TTC CTG GGC GAG TGG GGG CCC TTC CAG CGC CTC ATC TTC TTC CTG CTC 221
Phe Leu Gly Glu Trp Gly Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu
10 15 20 25

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AGC GCC AGC ATC ATC CCC AAT GGC TTC AAT GGT ATG TCA GTC GTG TTC      269
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                30                  35                  40
CTG GCG GGG ACC CCG GAG CAC CGC TGT CGA GTG CCG GAC GCC GCG AAC      317
Leu Ala Gly Thr Pro Glu His Arg Cys Arg Val Pro Asp Ala Ala Asn
            45                  50                  55
CTG AGC AGC GCC TGG CGC AAC AAC AGT GTC CCG CTG CGG CTG CGG GAC      365
Leu Ser Ser Ala Trp Arg Asn Asn Ser Val Pro Leu Arg Leu Arg Asp
        60                  65                  70
GGC CGC GAG GTG CCC CAC AGC TGC AGC CGC TAC CGG CTC GCC ACC ATC      413
Gly Arg Glu Val Pro His Ser Cys Ser Arg Tyr Arg Leu Ala Thr Ile
    75                  80                  85
GCC AAC TTC TCG GCG CTC GGG CTG GAG CCG GGG CGC GAC GTG GAC CTG      461
Ala Asn Phe Ser Ala Leu Gly Leu Glu Pro Gly Arg Asp Val Asp Leu
90                  95                  100                 105
GGG CAG CTG GAG CAG GAG AGC TGC CTG GAT GGC TGG GAG TTC AGC CAG      509
Gly Gln Leu Glu Gln Glu Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln
                110                 115                 120
GAC GTC TAC CTG TCC ACC GTC GTG ACC GAG TGG AAT CTG GTG TGT GAG      557
Asp Val Tyr Leu Ser Thr Val Val Thr Glu Trp Asn Leu Val Cys Glu
            125                 130                 135
GAC AAC TGG AAG GTG CCC CTC ACC ACC TCC CTG TTC TTC GTA GGC GTG      605
Asp Asn Trp Lys Val Pro Leu Thr Thr Ser Leu Phe Phe Val Gly Val
        140                 145                 150
CTC CTC GGC TCC TTC GTG TCC GGG CAG CTG TCA GAC AGG TTT GGC AGG      653
Leu Leu Gly Ser Phe Val Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg
    155                 160                 165
AAG AAC GTT CTC TTC GCA ACC ATG GCT GTA CAG ACT GGC TTC AGC TTC      701
Lys Asn Val Leu Phe Ala Thr Met Ala Val Gln Thr Gly Phe Ser Phe
170                 175                 180                 185
CTG CAG ATT TTC TCC ATC AGC TGG GAG ATG TTC ACT GTG TTA TTT GTC      749
Leu Gln Ile Phe Ser Ile Ser Trp Glu Met Phe Thr Val Leu Phe Val
                190                 195                 200
ATC GTG GGC ATG GGC CAG ATC TCC AAC TAT GTG GTA GCC TTC ATA CTA      797
Ile Val Gly Met Gly Gln Ile Ser Asn Tyr Val Val Ala Phe Ile Leu
            205                 210                 215
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GGA ACA GAA ATT CTT GGC AAG TCA GTT CGT ATT ATA TTC TCT ACA TTA      845
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        220                 225                 230
GGA GTG TGC ACA TTT TTT GCA GTT GGC TAT ATG CTG CTG CCA CTG TTT      893
Gly Val Cys Thr Phe Phe Ala Val Gly Tyr Met Leu Leu Pro Leu Phe
    235                 240                 245
GCT TAC TTC ATC AGA GAC TGG CGG ATG CTG CTG CTG GCG CTG ACG GTG      941
Ala Tyr Phe Ile Arg Asp Trp Arg Met Leu Leu Leu Ala Leu Thr Val
250                 255                 260                 265
CCG GGA GTG CTG TGT GTC CCG CTG TGG TGG TTC ATT CCT GAA TCT CCC      989
Pro Gly Val Leu Cys Val Pro Leu Trp Trp Phe Ile Pro Glu Ser Pro
                270                 275                 280
CGA TGG CTG ATA TCC CAG AGA AGA TTT AGA GAG GCT GAA GAT ATC ATC     1037
Arg Trp Leu Ile Ser Gln Arg Arg Phe Arg Glu Ala Glu Asp Ile Ile
            285                 290                 295
CAA AAA GCT GCA AAA ATG AAC AAC ACA GCT GTA CCA GCA GTG ATA TTT     1085
Gln Lys Ala Ala Lys Met Asn Asn Thr Ala Val Pro Ala Val Ile Phe
        300                 305                 310
GAT TCT GTG GAG GAG CTA AAT CCC CTG AAG CAG CAG AAA GCT TTC ATT     1133
Asp Ser Val Glu Glu Leu Asn Pro Leu Lys Gln Gln Lys Ala Phe Ile
    315                 320                 325
CTG GAC CTG TTC AGG ACT CGG AAT ATT GCC ATA ATG ACC ATT ATG TCT     1181
Leu Asp Leu Phe Arg Thr Arg Asn Ile Ala Ile Met Thr Ile Met Ser
330                 335                 340                 345
TTG CTG CTA TGG ATG CTG ACC TCA GTG GGT TAC TTT GCT CTG TCT CTG     1229
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                350                 355                 360
GAT GCT CCT AAT TTA CAT GGA GAT GCC TAC CTG AAC TGT TTC CTC TCT     1277
Asp Ala Pro Asn Leu His Gly Asp Ala Tyr Leu Asn Cys Phe Leu Ser
            365                 370                 375
GCC TTG ATT GAA ATT CCA GCT TAC ATT ACA GCC TGG CTG CTA TTG CGA     1325
Ala Leu Ile Glu Ile Pro Ala Tyr Ile Thr Ala Trp Leu Leu Leu Arg
        380                 385                 390
ACG CTG CCC AGG CGT TAT ATC ATA GCT GCA GTA CTG TTC TGG GGA GGA     1373
Thr Leu Pro Arg Arg Tyr Ile Ile Ala Ala Val Leu Phe Trp Gly Gly
    395                 400                 405
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410                 415                 420                 425
TCC ATT GGT CTG GTC ATG CTG GGA AAA TTT GGG ATC ACC TCT GCT TTC     1469
Ser Ile Gly Leu Val Met Leu Gly Lys Phe Gly Ile Thr Ser Ala Phe
                430                 435                 440
TCC ATG CTG TAT GTC TTC ACT GCT GAG CTC TAC CCA ACC CTG GTC AGG     1517
Ser Met Leu Tyr Val Phe Thr Ala Glu Leu Tyr Pro Thr Leu Val Arg
            445                 450                 455
AAC ATG GCG GTG GGG GTC ACA TCC ACG GCC TCC AGA GTG GGC AGC ATC     1565
Asn Met Ala Val Gly Val Thr Ser Thr Ala Ser Arg Val Gly Ser Ile
        460                 465                 470
ATT GCC CCC TAC TTT GTT TAC CTC GGT GCT TAC AAC AGA ATG CTG CCC     1613
Ile Ala Pro Tyr Phe Val Tyr Leu Gly Ala Tyr Asn Arg Met Leu Pro
    475                 480                 485
TAC ATC GTC ATG GGT AGT CTG ACT GTC CTG ATT GGA ATC TTC ACC CTT     1661
Tyr Ile Val Met Gly Ser Leu Thr Val Leu Ile Gly Ile Phe Thr Leu
490                 495                 500                 505
TTT TTC CCT GAA AGT TTG GGA ATG ACT CTT CCA GAA ACC TTA GAG CAG     1709
Phe Phe Pro Glu Ser Leu Gly Met Thr Leu Pro Glu Thr Leu Glu Gln
                510                 515                 520
ATG CAG AAA GTG AAA TGG TTC AGA TCT GGG AAA AAA ACA AGA GAC TCA     1757
Met Gln Lys Val Lys Trp Phe Arg Ser Gly Lys Lys Thr Arg Asp Ser
            525                 530                 535
ATG GAG ACA GAA GAA AAT CCC AAG GTT CTA ATA ACT GCA TTC             1799
Met Glu Thr Glu Glu Asn Pro Lys Val Leu Ile Thr Ala Phe
        540                 545                 550
TGAAAAAATA TCTACCCCAT TTGGTGAAGT GAAAAACAGA AAAATAAGAC CCTGTGGAGA   1859
AATTCGTTGT TCCCACTGAA ATGGACTGAC TGTAACGATT GACACCAAAA TGAACCTTGC   1919
TATCAAGAAA TGCTCGTCAT ACAGTAAACT CTGGATGATT CTTCCAGATA ATGTCCTTGC   1979
TTTACAAACC AACCATTTCT AGAGAGTCTC CTTACTCATT AATTCAATGA AATGGATTGG   2039
TAAGATGTCT TGAAAACATG TTAGTCAAGG ACTGGTAAAA TACATATAAA GATTAACACT   2099
CATTTCCAAT CATACAAATA CTATCCAAAT AAAAAT                              2135
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SEQ ID NO: 3

SEQUENCE LENGTH: 557

SEQUENCE TYPE: amino acid
TOPOLOGY: linear
MOLECULE TYPE: protein
SEQUENCE

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Asn Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu
        35                  40                  45
His Arg Cys Arg Val Pro Asp Ala Ala Asn Leu Ser Ser Ala Trp Arg
    50                  55                  60
Asn His Thr Val Pro Leu Arg Leu Arg Asp Gly Arg Glu Val Pro His
65                  70                  75
Ser Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Leu
80                  85                  90                  95
Gly Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Gln Leu Glu Gln Glu
                100                 105                 110
Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr
            115                 120                 125
Ile Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro
        130                 135                 140
Leu Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile
    145                 150                 155
Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val
160                 165                 170                 175
Thr Met Gly Met Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys
                180                 185                 190
Asn Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln
            195                 200                 205
Ile Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly
        210                 215                 220
Lys Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr
    225                 230                 235
Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp
240                 245                 250                 255
```

Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val
260 265 270
Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln
275 280 285
Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala
290 295 300
Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln
305 310 315
Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu
320 325 330 335
Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp
340 345 350
Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn
355 360 365
Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu
370 375 380
Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg
385 390 395
Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu
400 405 410 415
Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu
420 425 430
Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr
435 440 445
Val Tyr Thr Ala Glu Leu Tyr Pro Thr Val Val Arg Asn Met Gly Val
450 455 460
Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr
465 470 475
Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met
480 485 490 495
Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu
500 505 510
Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val
515 520 525
Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys
530 535 540

Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
545 550 555

SEQ ID NO: 4
SEQUENCE LENGTH: 1831
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: double
TOPOLOGY: linear
MOLECULE TYPE: cDNA to mRNA
SEQUENCE

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CACGCGCAAA GCCCGCCGCG TTCCCAGACC CCAGGCCGCG CTCTGTGGGC CTCTGAGGGC 120
GGC ATG CGG GAC TAC GAC GAG GTG ACC GCC TTC CTG GGC GAG TGG GGG 168
Met Arg Asp Tyr Asp Glu Val Thr Ala Phe Leu Gly Glu Trp Gly
1 5 10 15
CCC TTC CAG CGC CTC ATC TTC TTC CTG CTC AGC GCC AGC ATC ATC CCC 216
Pro Phe Gln Arg Leu Ile Phe Phe Leu Leu Ser Ala Ser Ile Ile Pro
20 25 30
AAT GGC TTC ACC GGC CTG TCC TCC GTG TTC CTG ATA GCG ACC CCG GAG 264
Asn Gly Phe Thr Gly Leu Ser Ser Val Phe Leu Ile Ala Thr Pro Glu
35 40 45
CAC CGC TGC CGG GTG CCG GAC GCC GCG AAC CTG AGC AGC GCC TGG CGC 312
His Arg Cys Arg Val Pro Asp Ala Ala Asn Leu Ser Ser Ala Trp Arg
50 55 60
AAC CAC ACT GTC CCA CTG CGG CTG CGG GAC GGC CGC GAG GTG CCC CAC 360
Asn His Thr Val Pro Leu Arg Leu Arg Asp Gly Arg Glu Val Pro His
65 70 75
AGC TGC CGC CGC TAC CGG CTC GCC ACC ATC GCC AAC TTC TCG GCG CTC 408
Ser Cys Arg Arg Tyr Arg Leu Ala Thr Ile Ala Asn Phe Ser Ala Leu
80 85 90 95
GGG CTG GAG CCG GGG CGC GAC GTG GAC CTG GGG CAG CTG GAG CAG GAG 456
Gly Leu Glu Pro Gly Arg Asp Val Asp Leu Gly Gln Leu Glu Gln Glu
100 105 110
AGC TGT CTG GAT GGC TGG GAG TTC AGT CAG GAC GTC TAC CTG TCC ACC 504
Ser Cys Leu Asp Gly Trp Glu Phe Ser Gln Asp Val Tyr Leu Ser Thr
115 120 125

```
ATT GTG ACC GAG TGG AAC CTG GTG TGT GAG GAC GAC TGG AAG GCC CCA      552
Ile Val Thr Glu Trp Asn Leu Val Cys Glu Asp Asp Trp Lys Ala Pro
        130                 135                 140
CTC ACA ATC TCC TTG TTC TTC GTG GGT GTG CTG TTG GGC TCC TTC ATT      600
Leu Thr Ile Ser Leu Phe Phe Val Gly Val Leu Leu Gly Ser Phe Ile
    145                 150                 155
TCA GGG CAG CTG TCA GAC AGG TTT GGC CGG AAG AAT GTG CTG TTC GTG      648
Ser Gly Gln Leu Ser Asp Arg Phe Gly Arg Lys Asn Val Leu Phe Val
160                 165                 170                 175
ACC ATG GGC ATG CAG ACA GGC TTC AGC TTC CTG CAG ATC TTC TCG AAG      696
Thr Met Gly Met Gln Thr Gly Phe Ser Phe Leu Gln Ile Phe Ser Lys
                180                 185                 190
AAT TTT GAG ATG TTT GTC GTG CTG TTT GTC CTT GTA GGC ATG GGC CAG      744
Asn Phe Glu Met Phe Val Val Leu Phe Val Leu Val Gly Met Gly Gln
            195                 200                 205
ATC TCC AAC TAT GTG GCA GCA TTT GTC CTG GGG ACA GAA ATT CTT GGC      792
Ile Ser Asn Tyr Val Ala Ala Phe Val Leu Gly Thr Glu Ile Leu Gly
        210                 215                 220
AAG TCA GTT CGT ATA ATA TTC TCT ACG TTA GGA GTG TGC ATA TTT TAT      840
Lys Ser Val Arg Ile Ile Phe Ser Thr Leu Gly Val Cys Ile Phe Tyr
    225                 230                 235
GCA TTT GGC TAC ATG GTG CTG CCA CTG TTT GCT TAC TTC ATC CGA GAC      888
Ala Phe Gly Tyr Met Val Leu Pro Leu Phe Ala Tyr Phe Ile Arg Asp
240                 245                 250                 255
TGG CGG ATG CTG CTG GTG GCG CTG ACG ATG CCG GGG GTG CTG TGC GTG      936
Trp Arg Met Leu Leu Val Ala Leu Thr Met Pro Gly Val Leu Cys Val
                260                 265                 270
GCA CTC TGG TGG TTC ATC CCT GAG TCC CCC CGA TGG CTC ATC TCT CAG      984
Ala Leu Trp Trp Phe Ile Pro Glu Ser Pro Arg Trp Leu Ile Ser Gln
            275                 280                 285
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Gly Arg Phe Glu Glu Ala Glu Val Ile Ile Arg Lys Ala Ala Lys Ala
        290                 295                 300
AAT GGG ATT GTT GTG CCT TCC ACT ATC TTT GAC CCG AGT GAG TTA CAA     1080
Asn Gly Ile Val Val Pro Ser Thr Ile Phe Asp Pro Ser Glu Leu Gln
    305                 310                 315
```

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Asp Leu Ser Ser Lys Lys Gln Gln Ser His Asn Ile Leu Asp Leu Leu
320                 325                 330                 335
CGA ACC TGG AAT ATC CGG ATG GTC ACC ATC ATG TCC ATA ATG CTG TGG      1176
Arg Thr Trp Asn Ile Arg Met Val Thr Ile Met Ser Ile Met Leu Trp
                340                 345                 350
ATG ACC ATA TCA GTG GGC TAT TTT GGG CTT TCG CTT GAT ACT CCT AAC      1224
Met Thr Ile Ser Val Gly Tyr Phe Gly Leu Ser Leu Asp Thr Pro Asn
            355                 360                 365
TTG CAT GGG GAC ATC TTT GTG AAC TGC TTC CTT TCA GCG ATG GTT GAA      1272
Leu His Gly Asp Ile Phe Val Asn Cys Phe Leu Ser Ala Met Val Glu
        370                 375                 380
GTC CCA GCA TAT GTG TTG GCC TGG CTG CTG CTG CAA TAT TTG CCC CGG      1320
Val Pro Ala Tyr Val Leu Ala Trp Leu Leu Leu Gln Tyr Leu Pro Arg
    385                 390                 395
CGC TAT TCC ATG GCC ACT GCC CTC TTC CTG GGT GGC AGT GTC CTT CTC      1368
Arg Tyr Ser Met Ala Thr Ala Leu Phe Leu Gly Gly Ser Val Leu Leu
400                 405                 410                 415
TTC ATG CAG CTG GTA CCC CCA GAC TTG TAT TAT TTG GCT ACA GTC CTG      1416
Phe Met Gln Leu Val Pro Pro Asp Leu Tyr Tyr Leu Ala Thr Val Leu
                420                 425                 430
GTG ATG GTG GGC AAG TTT GGA GTC ACG GCT GCC TTT TCC ATG GTC TAC      1464
Val Met Val Gly Lys Phe Gly Val Thr Ala Ala Phe Ser Met Val Tyr
            435                 440                 445
GTG TAC ACA GCC GAG CTG TAT CCC ACA GTG GTG AGA AAC ATG GGT GTG      1512
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Gly Val Ser Ser Thr Ala Ser Arg Leu Gly Ser Ile Leu Ser Pro Tyr
    465                 470                 475
TTC GTT TAC CTT GGT GCC TAC GAC CGC TTC CTG CCC TAC ATT CTC ATG      1608
Phe Val Tyr Leu Gly Ala Tyr Asp Arg Phe Leu Pro Tyr Ile Leu Met
480                 485                 490                 495
GGA AGT CTG ACC ATC CTG ACA GCC ATC CTC ACC TTG TTT CTC CCA GAG      1656
Gly Ser Leu Thr Ile Leu Thr Ala Ile Leu Thr Leu Phe Leu Pro Glu
                500                 505                 510
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AGC TTC GGT ACC CCA CTC CCA GAC ACC ATT GAC CAG ATG CTA AGA GTC 1704
Ser Phe Gly Thr Pro Leu Pro Asp Thr Ile Asp Gln Met Leu Arg Val
515 520 525
AAA GGA ATG AAA CAC AGA AAA ACT CCA AGT CAC ACA AGG ATG TTA AAA 1752
Lys Gly Met Lys His Arg Lys Thr Pro Ser His Thr Arg Met Leu Lys
530 535 540
GAT GGT CAA GAA AGG CCC ACA ATC CTT AAA AGC ACA GCC TTC 1794
Asp Gly Gln Glu Arg Pro Thr Ile Leu Lys Ser Thr Ala Phe
545 550 555
TAACATCGCT TCCAGTAAGG GAGAAACTGA AGAGGAA 1831

SEQ ID NO: 5
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
CTAATACGAC TCACTATAGG GC 22

SEQ ID NO: 6
SEQUENCE LENGTH: 21
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 7
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

TCGAGCGGCC GCCCGGGCAG GT 22

SEQ ID NO: 8
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
AGGGCGTGGT GCGGAGGGCG GT 22

SEQ ID NO: 9
SEQUENCE LENGTH: 20
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
CTTTTGAGCA AGTTCAGCCT 20

SEQ ID NO: 10
SEQUENCE LENGTH: 24
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 11
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

CCAGGGTTTT CCCAGTCACG AC 22

SEQ ID NO: 12
SEQUENCE LENGTH: 22
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
TCACACAGGA AACAGCTATG AC 22

SEQ ID NO: 13
SEQUENCE LENGTH: 24
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 14
SEQUENCE LENGTH: 24
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 15
SEQUENCE LENGTH: 24
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
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AGCATCCTGT CTCCCTACTT CGTT 24

SEQ ID NO: 16
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SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
GATGGATCCC GGACGGTCTT GGGTCGCCTG CTG 33

SEQ ID NO: 17
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 18
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE
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SEQ ID NO: 19
SEQUENCE LENGTH: 33
SEQUENCE TYPE: nucleic acid
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: other nucleic acid, synthetic DNA
SEQUENCE

GATGGATCCT TCCTCTTCAG TTTCTCCCTT ACT 33

[Brief Description of the Drawings]

[Figure 1] It represents hydrophobicity plots of OCTN1 and OCTN2 according to Kyte & Doolittle's calculating formula with a window of nine amino acid residues. Numerals on the plots indicate putative transmembrane regions.

[Figure 2] It represents electrophoretic patterns showing the results of Northern blot analysis of OCTN1.

[Figure 3] It compares the amino acid sequence of OCTN1 with that of OCTN2. Amino acid residues conserved in both transporters are shaded. Sequences coinciding with the consensus sequences of sugar transporter and the ATP/GTP binding site are indicated by "+" and "*," respectively.

[Figure 4] It represents electrophoretic patterns showing the results of Northern blot analysis of OCTN2.

[Figure 5] It is a graph showing the TEA-absorbing activity of OCTN1. Clear circles represent untreated cells, and solid circles represent OCTN1-transfected cells.

[Figure 6] It is a graph showing effects of the cold TEA added in the experimental system in Fig. 5. In this graph, solid circles represent OCTN1-transfected cells, and clear circles represent cells containing the vector with no insert. Clear triangles indicate the net uptake induced by OCTN1 obtained by subtracting the clear circle values from the corresponding solid circle values.

[Figure 7] It is a graph showing TEA concentration-dependency of the TEA-absorbing activity of OCTN1.

[Figure 8] It is a bar graph showing the activity of the OCTN1-transfected cells to absorb substances other than TEA.

[Figure 9] It is a bar graph showing the results of transport experiments using *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the OCTN1-injected cRNA oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of TEA, carnitine, mepyramine, quinidine, and actinomycin D were observed in OCTN1 cRNA-injected oocytes, whereas water-injected oocytes (containing no cRNA)

exhibited almost no uptake activity.

[Figure 10] It is a bar graph showing the results of transport experiments for carcinostatics in *Xenopus* oocytes. Bars indicated with "OCTN1" and "Water" represent the uptake activity of the OCTN1 cRNA-injected oocytes and that of the water-injected oocytes (containing no cRNA), respectively. Uptakes of actinomycin D, etoposide, vinblastine, and daunomycin were observed in the OCTN1 cRNA-injected oocytes.

[Figure 11] It is a bar graph showing the results of transport experiments with OCTN1 and OCTN2 in HEK293 cells. OCTN1 has the efficient transport activity for TEA and OCTN2 for carnitine.

[Figure 12] It is a graph showing the results of Na^+-dependency of the carnitine transport activity of OCTN2. OCTN2 exhibits a time-dependent carnitine transport activity (clear circle) in the presence of Na^+, while no such activity in the absence of Na^+ (solid circle), indicating that the carnitine transport activity of OCTN2 depends on the presence of Na^+.

Drawings

Figure 1

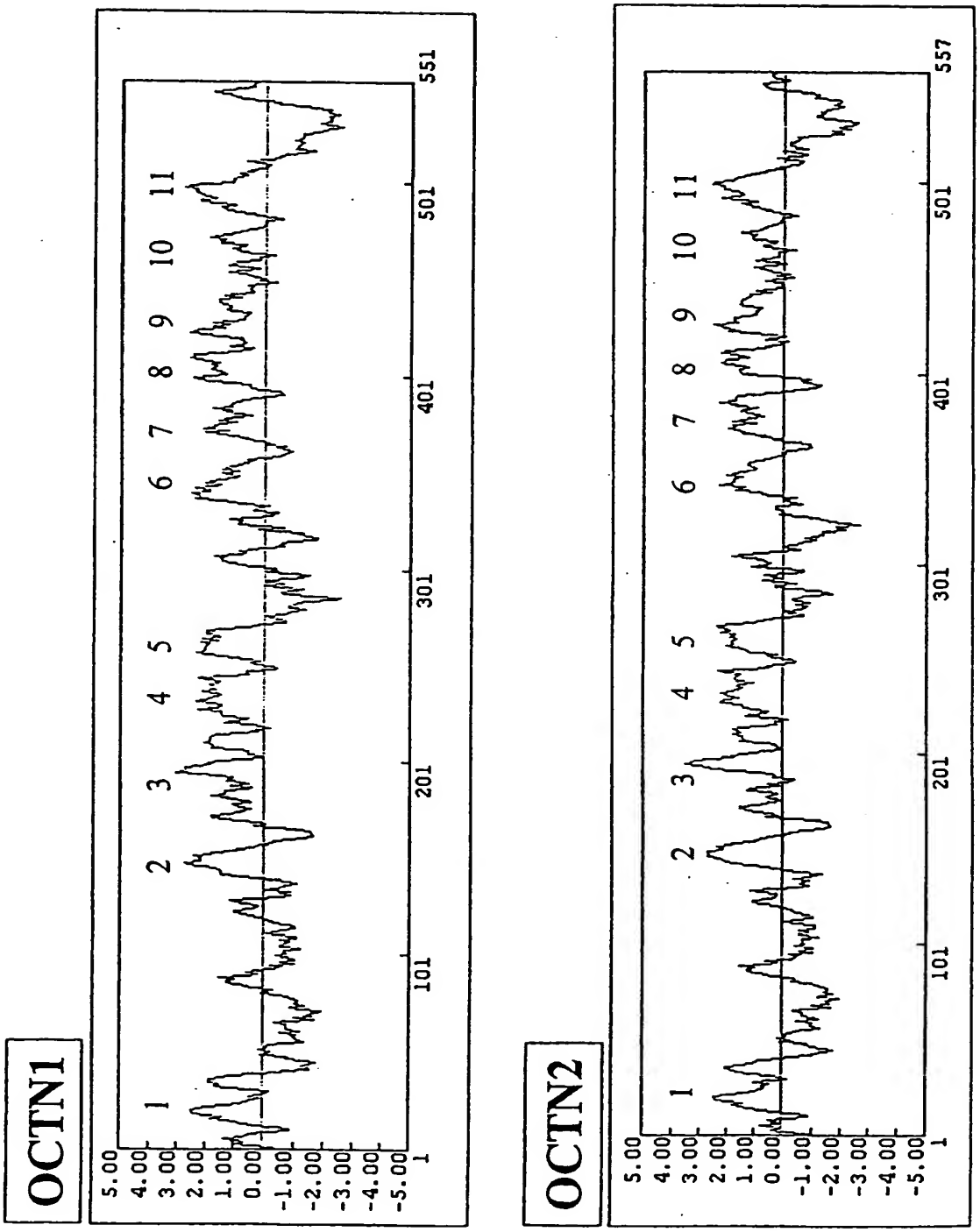
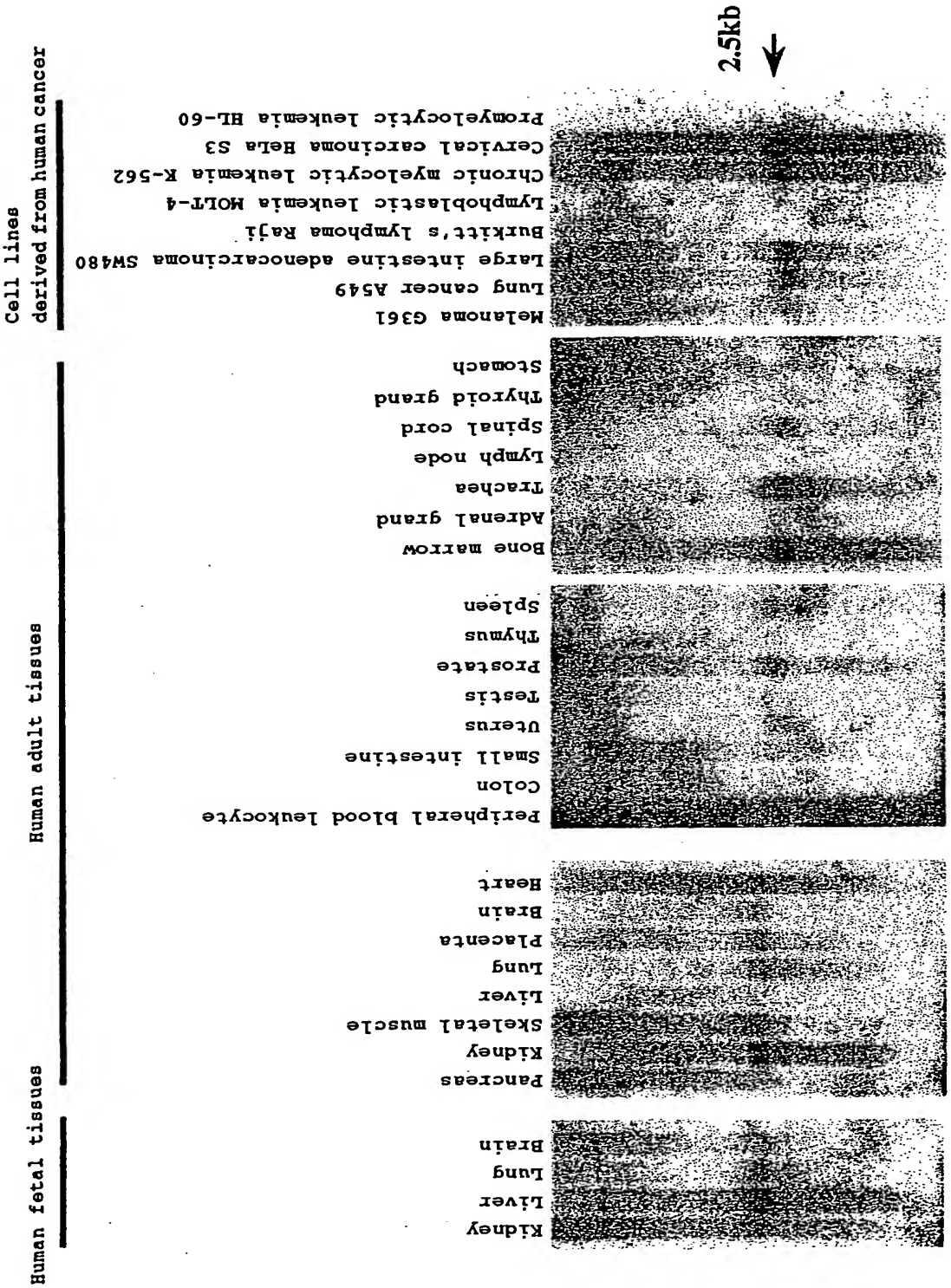


Figure 2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Cell lines derived from human cancer
Melanoma G361
Lung cancer A549
Large intestine adenocarcinoma SW480
Burkitt's lymphoma Raji
Lymphoblastic leukemia MOLT-4
Chronic myelocytic leukemia K-562
Cervical carcinoma HeLa S3
Promyelocytic leukemia HL-60
Human adult tissues
Bone marrow
Adrenal grand
Trachea
Lymph node
Spinal cord
Thyroid grand
Stomach
Peripheral blood leukocyte
Colon
Small intestine
Uterus
Testis
Prostate
Thymus
Spleen
Pancreas
Kidney
Skeletal muscle
Liver
Lung
Placenta
Brain
Heart
Human fetal tissues
Kidney
Liver
Lung
Brain

Figure 3

Figure 4

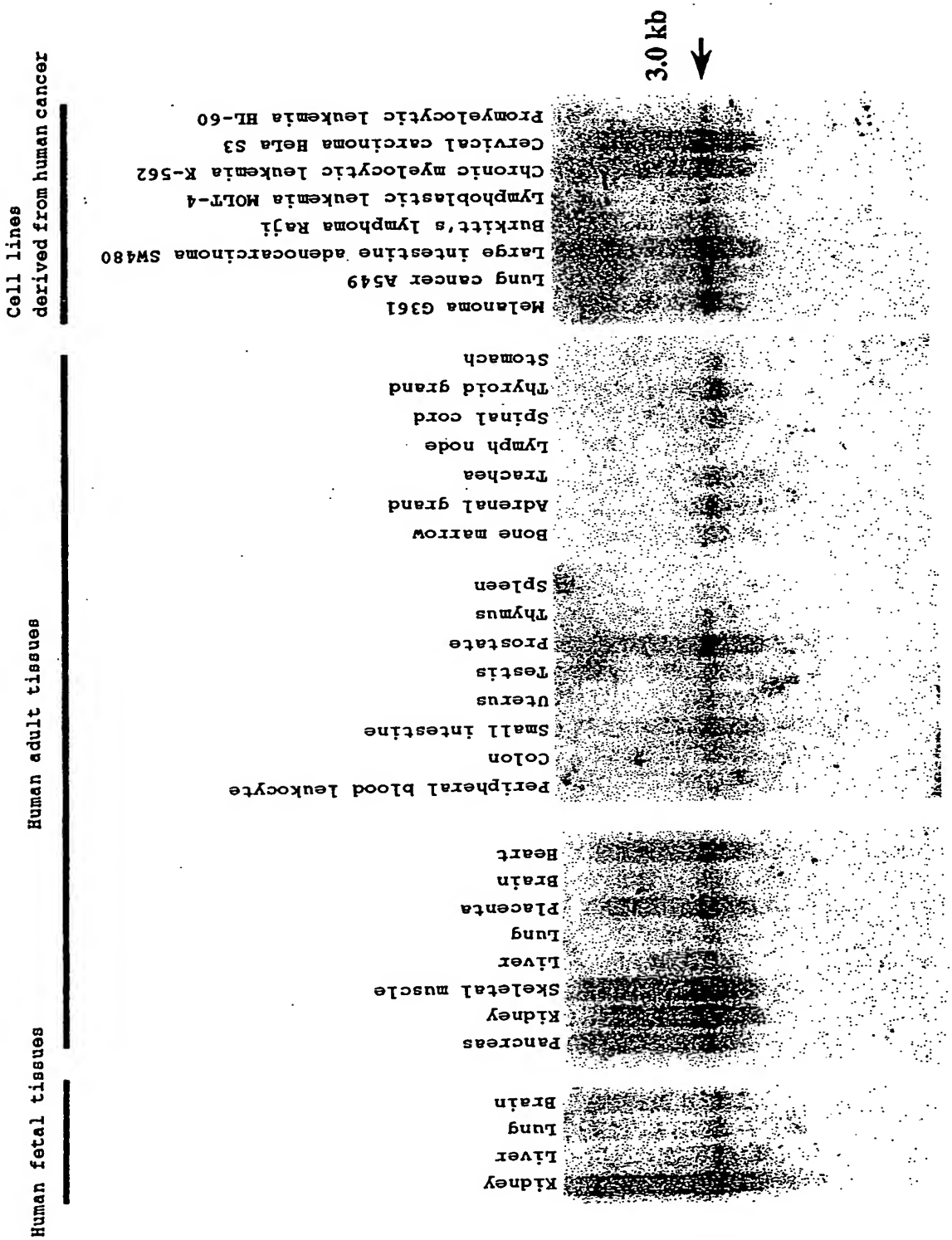

3.0 kb
Cell lines derived from human cancer
Melanoma G361
Lung cancer A549
Large intestine adenocarcinoma SW480
Burkitt's lymphoma Raji
Lymphoblastic leukemia MOLT-4
Chronic myelocytic leukemia K-562
Cervical carcinoma HeLa S3
Promyelocytic leukemia HL-60
Human adult tissues
Stomach
Thyroid grand
Spinal cord
Lymph node
Trachea
Adrenal grand
Bone marrow
Spleen
Thymus
Prostate
Testis
Uterus
Small intestine
Colon
Peripheral blood leukocyte
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
Human fetal tissues
Brain
Lung
Liver
Kidney

Figure 5

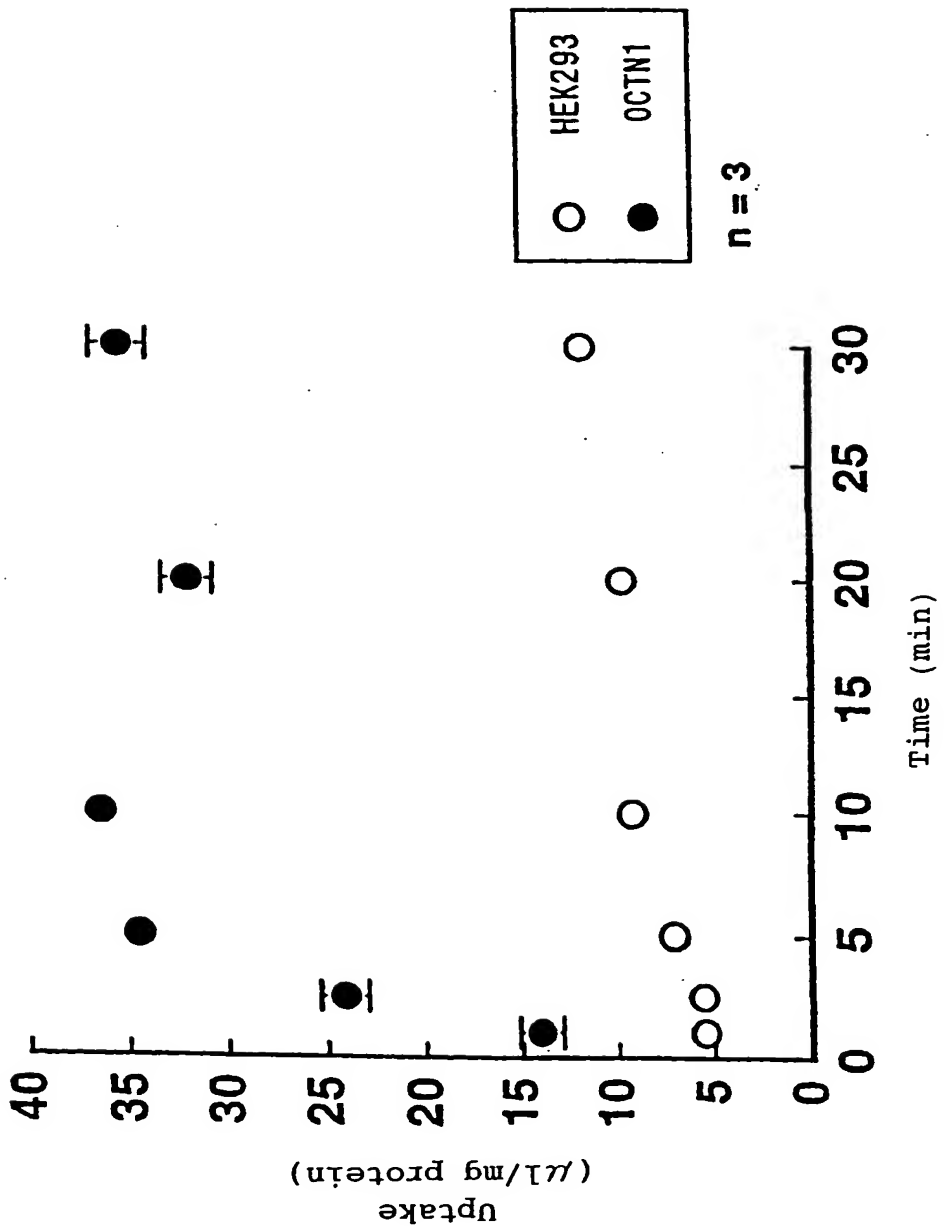

HEK293
OCTN1
n = 3
uptake
(μl/mg protein)
0
5
10
15
20
25
30
35
40
0
5
10
15
20
25
30
Time (min)

Figure 6

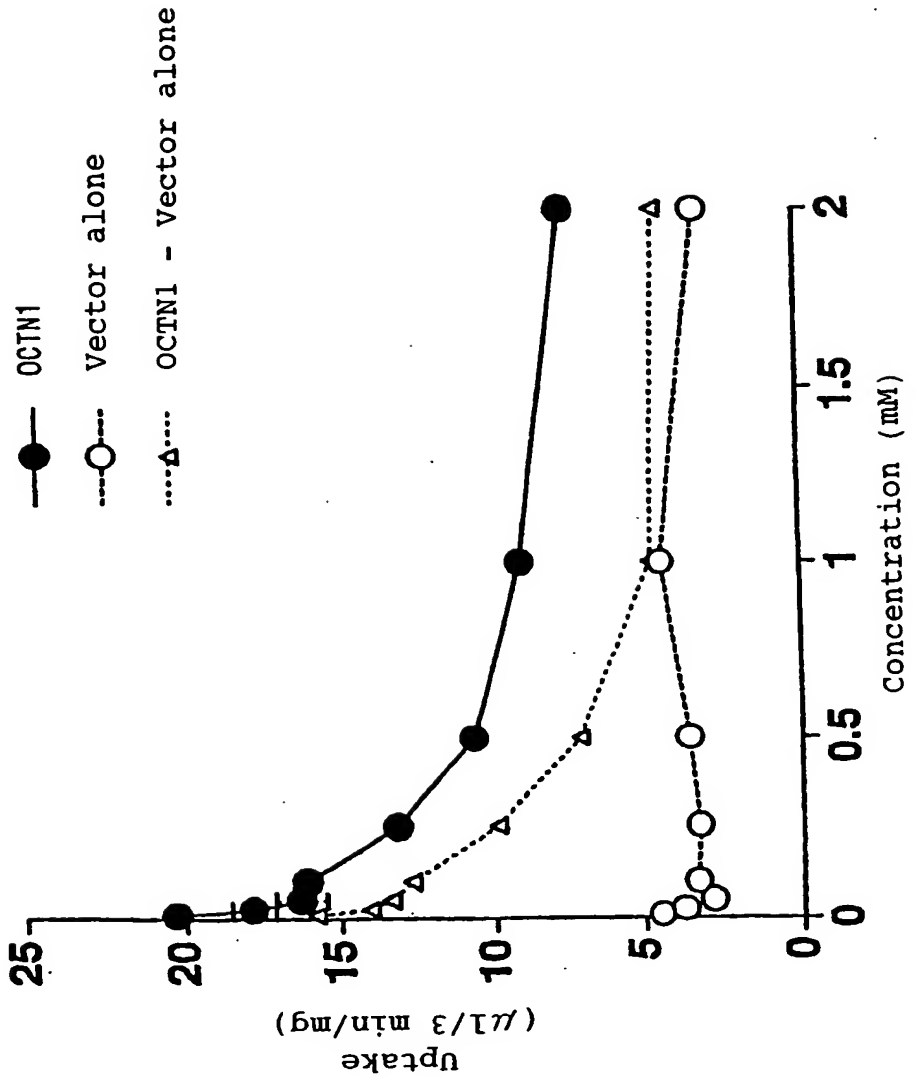

OCTN1
Vector alone
OCTN1 - Vector alone
uptake
(μl/3 min/mg)
25
20
15
10
5
0
0
0.5
1
1.5
2
Concentration (mM)

Figure 7

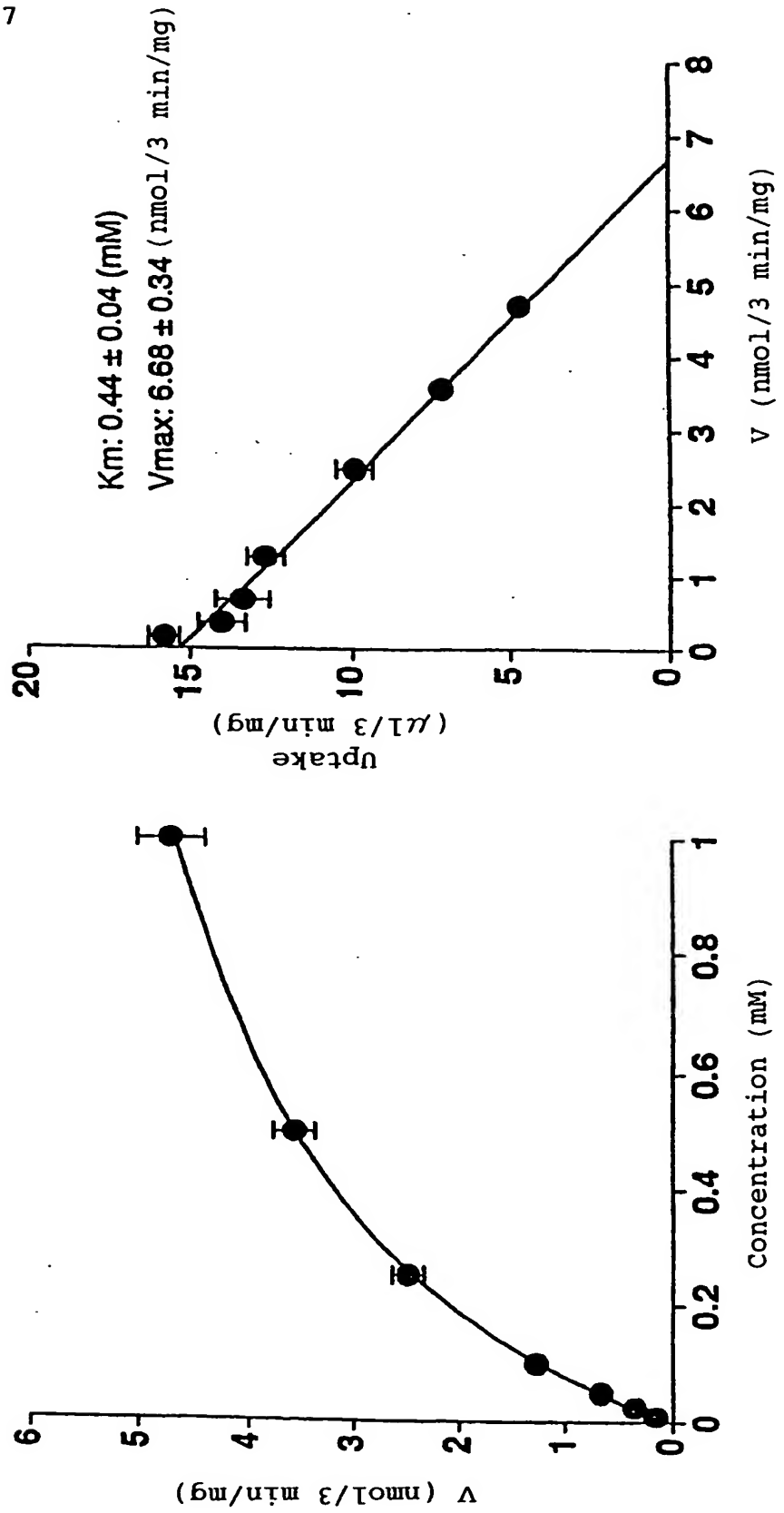

Km: 0.44 ± 0.04 (mM)
Vmax: 6.68 ± 0.34 (nmol/3 min/mg)
Uptake (μl/3 min/mg)
V (nmol/3 min/mg)
V (nmol/3 min/mg)
Concentration (mM)

Figure 8

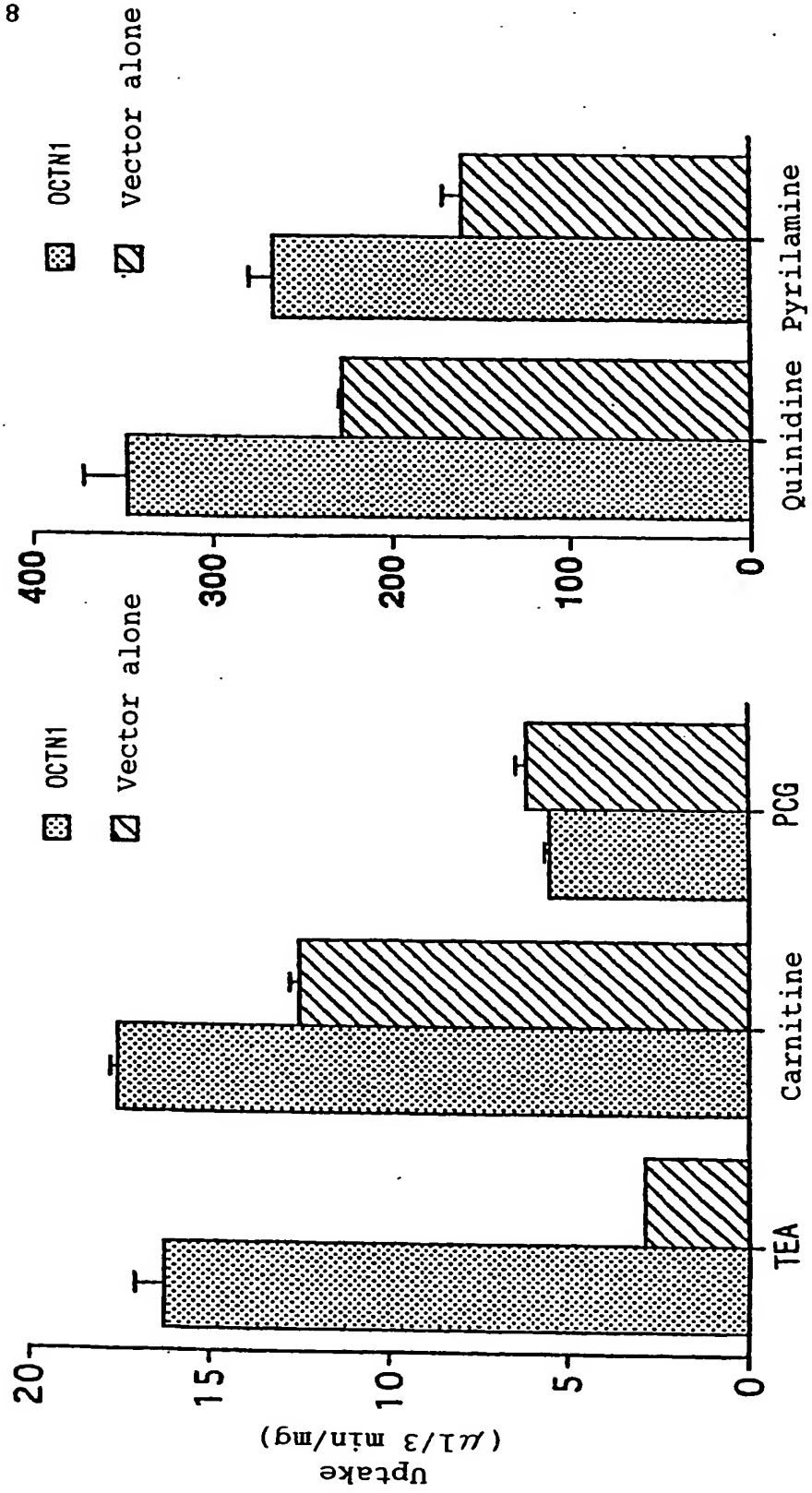

OCTN1
Vector alone
Uptake
(μl/3 min/mg)
0
5
10
15
20
TEA
Carnitine
PCG
0
100
200
300
400
Quinidine
Pyrilamine

Figure 9

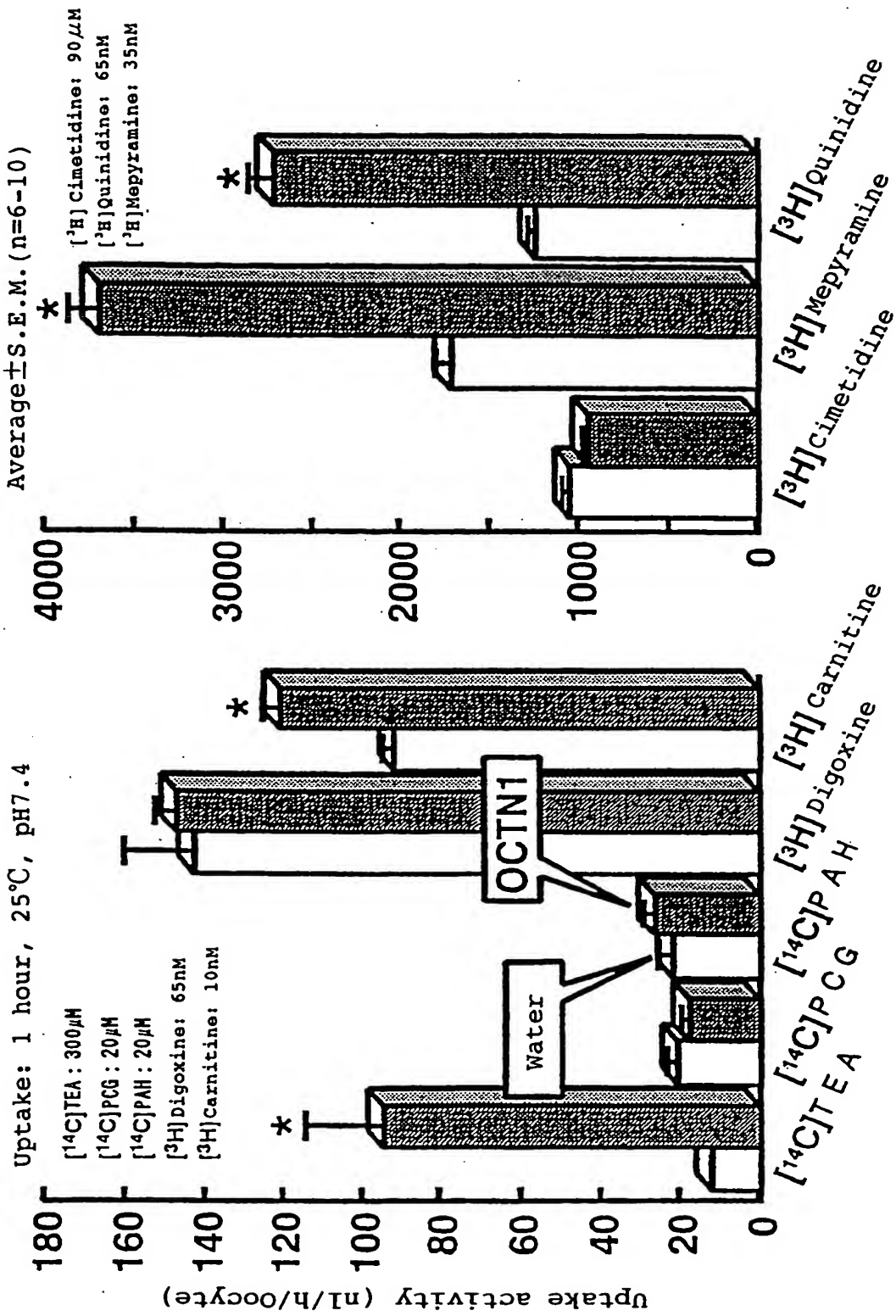

Uptake: 1 hour, 25°C, pH7.4
[14C]TEA : 300μM
[14C]PCG : 20μM
[14C]PAH : 20μM
[3H]Digoxine: 65nM
[3H]Carnitine: 10nM
Water
OCTN1
Uptake activity (nl/h/oocyte)
Average±S.E.M.(n=6-10)
[3H]Cimetidine: 90μM
[3H]Quinidine: 65nM
[3H]Mepyramine: 35nM
[14C]TEA
[14C]PCG
[14C]PAH
[3H]Digoxine
[3H]Carnitine
[3H]Cimetidine
[3H]Mepyramine
[3H]Quinidine

Figure 10

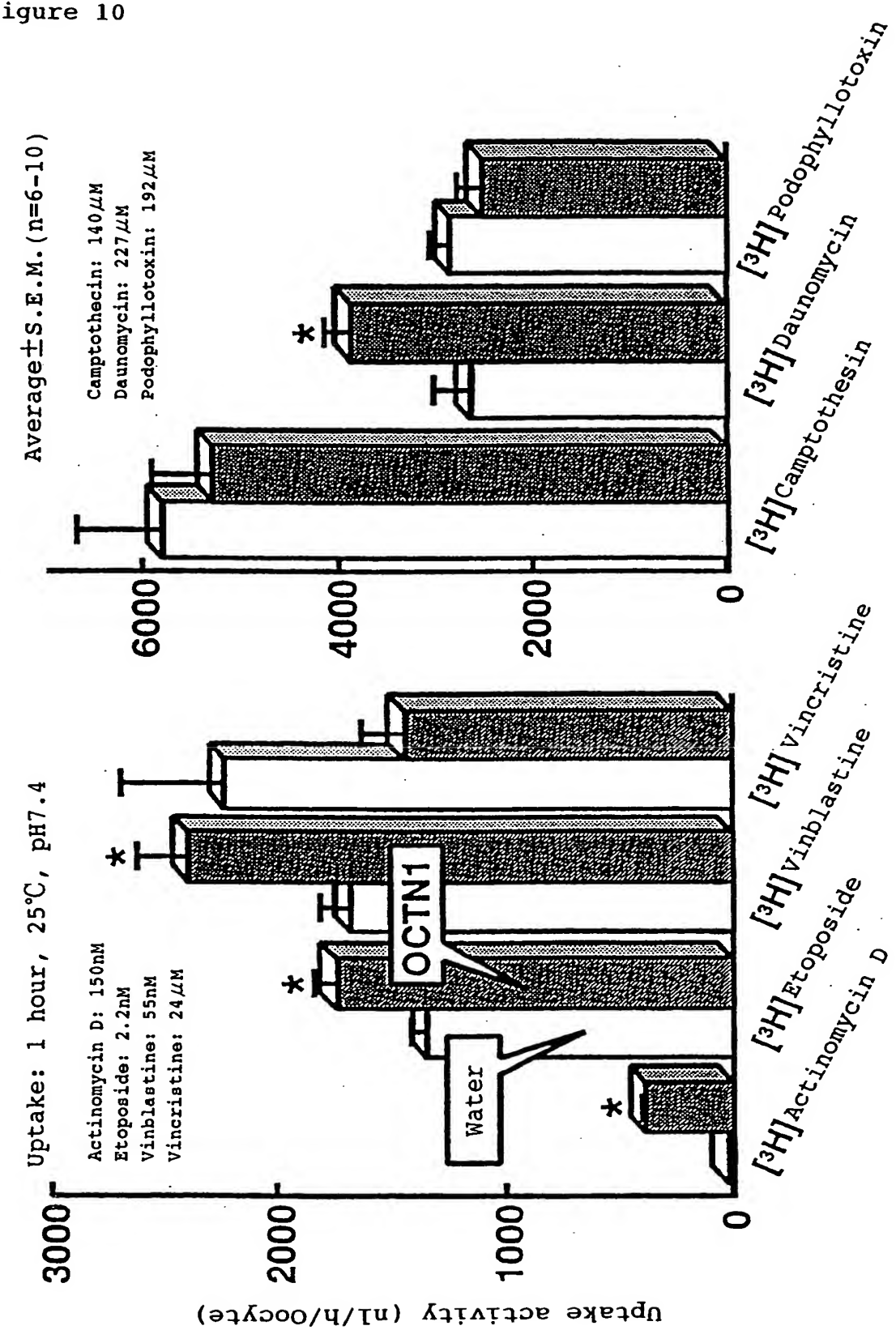
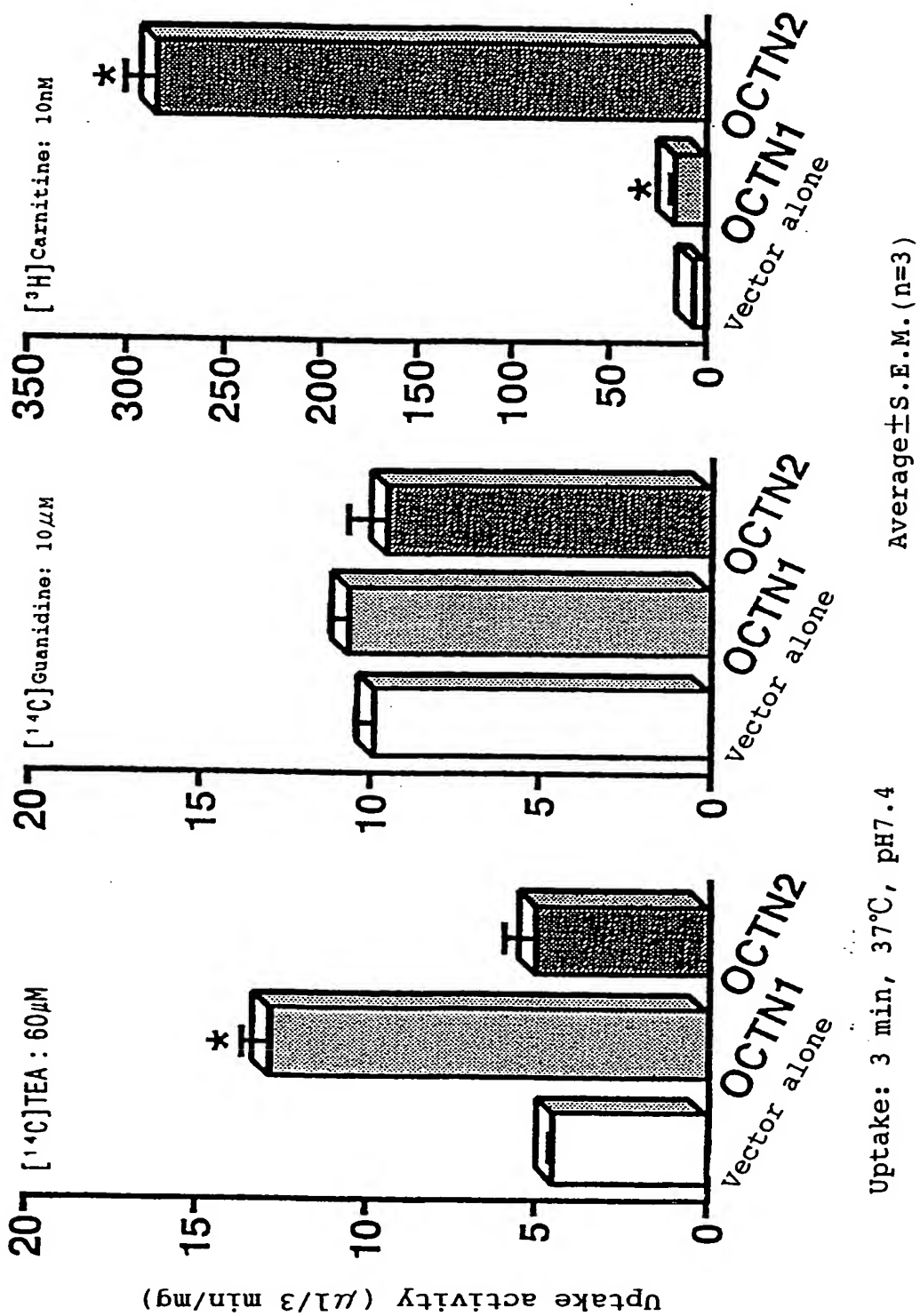
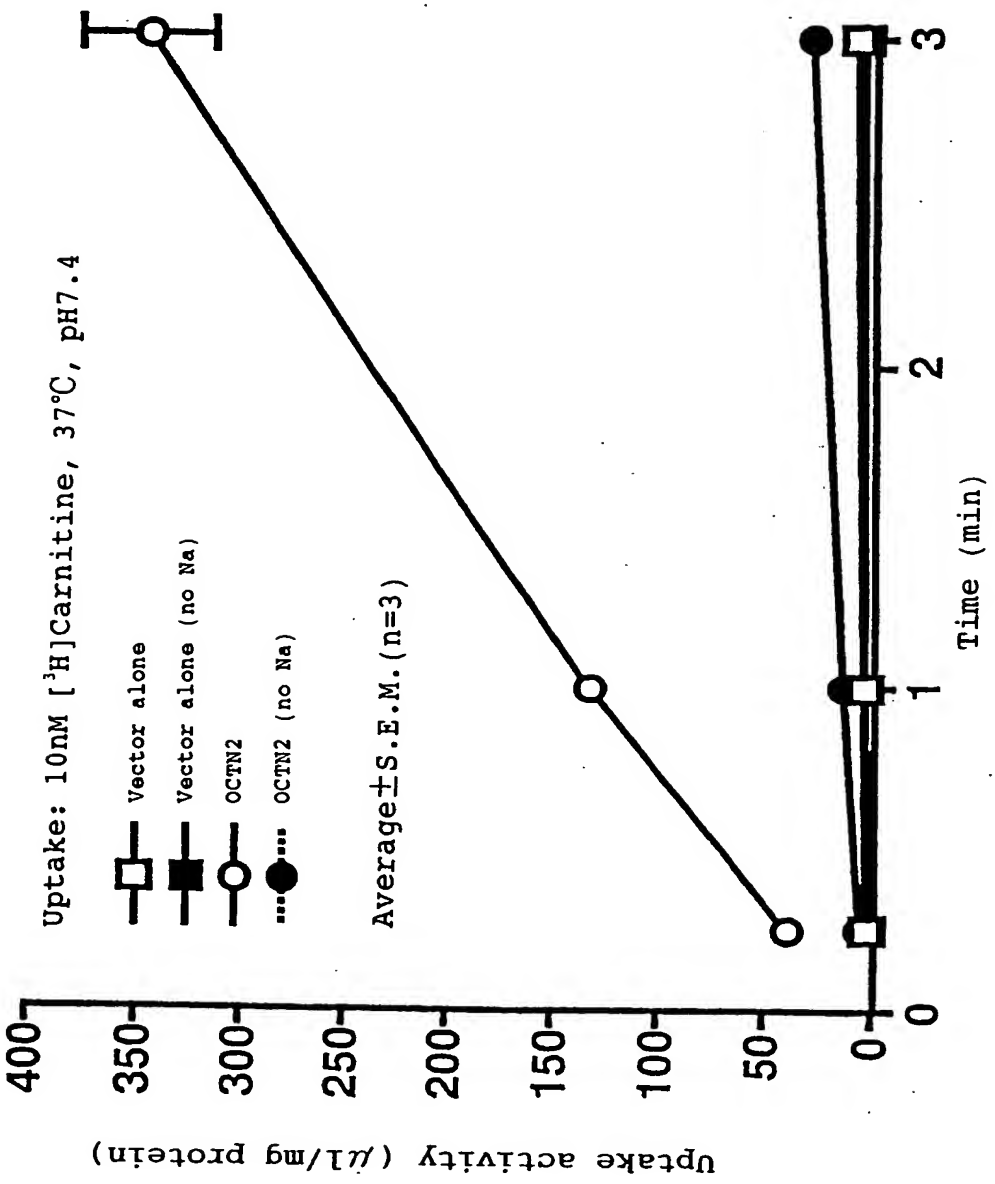

Uptake: 1 hour, 25°C, pH7.4
Actinomycin D: 150nM
Etoposide: 2.2nM
Vinblastine: 55nM
Vincristine: 24μM
Average±S.E.M.(n=6-10)
Camptothecin: 140μM
Daunomycin: 227μM
Podophyllotoxin: 192μM
Water
OCTN1
Uptake activity (nl/h/oocyte)
3000
2000
1000
0
6000
4000
2000
0
[3H]Actinomycin D
[3H]Etoposide
[3H]Vinblastine
[3H]Vincristine
[3H]Camptothesin
[3H]Daunomycin
[3H]Podophyllotoxin

Figure 11


[14C]TEA : 60μM
[14C]Guanidine: 10μM
[3H]Carnitine: 10nM
Uptake activity (μl/3 min/mg)
Vector alone
OCTN1
OCTN2
Uptake: 3 min, 37°C, pH7.4
Average±S.E.M.(n=3)

Reference No. = C2-906DP1

Figure 12



[Document Name] Abstract

[Abstract]

[Problems to Be Solved] An objective of the present invention is to provide a novel transporter gene and its protein.

[Means to Solve the Problems] An unknown gene significantly homologous to organic cation transporters OCT1 and OCT2 has been successfully isolated by screening a fetal gene library using random sequencing. Since it was expected that the gene would encode a novel transporter, inventors succeeded in isolating the desired gene by screening the cDNA library derived from human fetus. As a result of studying the transporter activity of the protein encoded by the isolated gene, it was revealed that the protein functions as transporters of various organic cations.

[Selected Drawing] None